

1 **The importance of replicating genomic analyses to verify phylogenetic signal for**
2 **recently-evolved lineages.**

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25 **Running title:** Resolving closely-related species using SNPs

26 **Abstract:**

27 Genome-wide SNP data generated by non-targeted methods such as RAD and GBS are
28 increasingly being used in phylogenetic and phylogeographic analyses. When these methods
29 are used in the absence of a reference genome, however, little is known about the locations
30 and evolution of the SNPs. In using such data to address phylogenetic questions, researchers
31 risk drawing false conclusions, particularly if a representative number of SNPs is not
32 obtained. Here, we empirically test the robustness of phylogenetic inference based on SNP
33 data for closely-related lineages. We conducted a genome-wide analysis of 75,712 SNPs,
34 generated via GBS, of southern bull-kelp (*Durvillaea*). *Durvillaea chathamensis* co-occurs
35 with *D. antarctica* on Chatham Island, but the two species have previously been found to be
36 so genetically similar that the status of the former has been questioned. Our results show that
37 *D. chathamensis*, which differs from *D. antarctica* ecologically as well as morphologically, is
38 indeed a reproductively isolated species. Furthermore, our replicated analyses show that *D.*
39 *chathamensis* cannot be reliably distinguished phylogenetically from closely-related *D.*
40 *antarctica* using subsets (ranging in size from 400 to 40,912 sites) of the parsimony-
41 informative SNPs in our dataset, and that bootstrap values alone can give misleading
42 impressions of the strength of phylogenetic inferences. These results highlight the importance
43 of independently replicating SNP analyses to verify that phylogenetic inferences based on
44 non-targeted SNP data are robust. Our study also demonstrates that modern genomic
45 approaches can be used to identify cases of recent or incipient speciation that traditional
46 approaches (e.g., Sanger sequencing of a few loci) may be unable to detect or resolve.

47 **Introduction**

48 High-throughput DNA sequencing technologies are becoming increasingly popular for
49 phylogenetic analysis. For non-model organisms, obtaining large amounts of genomic data
50 for phylogenetic analysis has, however, proven challenging, as tools such as universal
51 primers have only been developed for relatively few phylogenetically-informative regions
52 (Faircloth et al. 2012), and targeted enrichment approaches such as exon capture require
53 knowledge of the genome of the study taxon (Faircloth et al. 2012; Hugall et al. 2016) or
54 close relatives (Bragg et al. 2015). In contrast, SNP data generated by non-targeted methods
55 such as RAD tag (Restriction-site-Associated DNA tags: Miller et al. 2007; Baird et al. 2008)
56 and GBS (Genotyping-by-Sequencing: Elshire et al. 2011) offer appealing alternatives to
57 targeted phylogenetic methods, including for species delimitation (Bryant et al. 2012; Leaché
58 et al. 2014; Herrera and Shank 2015; Pante et al. 2015), as large amounts of data – tens to
59 hundreds of thousands of SNPs – can be obtained with no prior knowledge of the genome.
60 Genomic analyses of closely-related sympatric lineages can, for example, help to detect
61 neofascinating but reproductively isolated species, shedding light on the evolutionary processes
62 driving speciation where traditional methods such as sequencing a few, targeted loci might
63 fail (Pante et al. 2015).

64

65 When SNPs are obtained by non-targeted methods, and in the absence of a reference genome,
66 little or nothing is known about the genomic locations of the SNPs, precluding inference of
67 their positional-based evolutionary dynamics, and thus potentially violating the assumptions
68 of downstream phylogenetic analyses. Among-site rate variation in a genome can, for
69 example, be substantial: in mitochondrial genomes, some sites have been inferred to have
70 evolved up to 1000 times faster than others (e.g., Galtier et al. 2006; Kjer and Honeycutt
71 2007; Rosset et al. 2008; Song et al. 2010). Such rate variation can lead to biased estimation
72 of branch lengths, with large impacts on the accuracy of phylogeny estimation, substitution
73 rates, and evolutionary divergence estimates (e.g., Wakeley 1993; Tateno et al. 1994; Yang
74 1996; Buckley et al. 2001; Sullivan and Swofford 2001; Simon et al. 2006; Soubrier et al.
75 2012). In datasets generated using random restriction enzyme digests and without a reference
76 genome, SNP sites will correspond to a range of unknown, divergent genomic locations,
77 including coding and non-coding regions; applying appropriate evolutionary models to
78 account for heterogeneity in mutation rates among sites in phylogenetic analyses using *a*
79 *priori* knowledge of the site characteristics is thus not possible without annotating gene

80 fragments using genomic data from other species. Such shortcomings could theoretically be
81 offset by using a sufficiently large number of SNPs, but the number of parsimony-
82 informative SNPs – which will be a subset of any dataset – needed to provide an accurate
83 phylogeny, is not clear. In a recent study using shotgun sequencing of gibbon (Hylobatidae)
84 genomes, taxa could be distinguished as effectively using 25,531 SNPs as with random
85 subsets of 200 SNPs (Veeramah et al. 2015), but these analyses were largely looking at deep
86 (intergeneric) divergences which were not always well resolved even using the full dataset;
87 how well small datasets of genome-wide SNPs can resolve relationships of recently-diverged
88 taxa and / or populations remains to be determined.

89
90 Geologically recent islands provide ideal natural laboratories for studying speciation
91 processes (Shaw 1996; Mendelson and Shaw 2005). Chatham Island, a small (920 km²)
92 island situated 650 km east of mainland New Zealand, emerged within the last few million
93 years (Campbell 2008; Heenan et al. 2010), and houses a distinctive biota largely assembled
94 via trans-oceanic dispersal events from mainland New Zealand source populations (Trewick
95 2000; Goldberg et al. 2008; Heenan et al. 2010; Goldberg and Trewick 2011). The bull-kelp
96 *Durvillaea chathamensis* (Hay 1979a) co-occurs on the island with a widespread congeneric
97 *D. antarctica*. *Durvillaea chathamensis* is non-buoyant and is endemic to Chatham Island,
98 whereas *D. antarctica* is both buoyant and widespread, dominating many rocky shore
99 ecosystems in the Southern Hemisphere, and dispersing long distances via rafting (Fraser et
100 al. 2009; Fraser et al. 2011). The validity of *D. chathamensis* has been questioned based on
101 cladistic analyses (Cheshire et al. 1995), and recent molecular analyses of the genus (Fig. 1)
102 (Fraser et al. 2010) also failed to provide strong evidence in support of its status as a distinct
103 species. Indeed, this latter work revealed a close phylogenetic relationship between *D.*
104 *chathamensis* and a northern New Zealand clade of *D. antarctica*, including shared alleles at
105 cytoplasmic and nuclear loci (Fig. 1) (Fraser et al. 2010). The two species, which grow side-
106 by-side in the Chatham Island intertidal (Schiel et al. 1995), are nonetheless morphologically
107 distinct (Hay 1979b) and have slightly different ecological niches: *D. antarctica* is hollow-
108 bladed and grows only intertidally, from mid- to low-tide mark, whereas *D. chathamensis* is
109 solid-bladed and mainly occurs sub-tidally, from the low-tide mark to about two metres depth
110 (Hay 1979b). We hypothesized that the two morphotypes represent a case of recent
111 speciation, with isolation of the lineages having occurred too recently to be detectable using
112 traditional Sanger sequencing of standard loci used in phylogenetic analyses (such as *cox1*,

113 28S, 18S). Here we use GBS data to: i) assess the genealogical basis for separate recognition
114 of *D. antarctica* and *D. chathamensis*; and ii) assess how many SNPs are needed for our
115 phylogenetic conclusions to be considered robust.

116

117 **Methods**

118 *Sampling*

119 Samples of sympatric *D. antarctica* (n = 23) and *D. chathamensis* (n = 27) were collected
120 from three Chatham Island intertidal localities (Fig. 1, Table 1) at which these taxa grow side
121 by side. In addition, *D. antarctica* samples from five mainland NZ localities (n = 19) —
122 focusing specifically on nearby localities that show particularly close phylogenetic similarity
123 with the Chathams *Durvillaea* assemblage for mtDNA markers (Fraser et al. 2010) (Fig. 1)
124 — and from sub-Antarctic Marion Island (n = 4) and the Falkland Islands (n = 7), were
125 included. Tissue samples were preserved in the field in 96% ethanol, and later dried at 60°C
126 for several hours before being placed in ziplock bags containing silica gel beads.

127

128 *DNA Extraction*

129 DNA was extracted using the MoBio PowerPlant Pro kit (MoBio, Carlsbad, CA). Brown
130 algal (phaeophycean) tissue can contain polysaccharides that interfere with PCR and DNA
131 digestion, and initial screening of extractions indicated low-purity DNA, so modifications to
132 the extraction protocol were made, as follows. A small (~ 1 mm²) fragment of dried kelp
133 tissue was softened by soaking in 400 µl dH₂O for two hours at 60°C. Samples were then
134 vortexed in tubes containing steel beads for up to two minutes. PowerPlant PD1, PD2 and
135 RNase A solutions were added according to the manufacturers' instructions. Samples were
136 vortexed briefly, incubated at 65°C for ten minutes, and vortexed again for up to two
137 minutes. 100 µl isopropanol was added to limit precipitation of DNA. Subsequent steps were
138 as per manufacturers' protocols, with final elution in 50 µl PD7 solution. Extracted DNA
139 appeared to still contain some alginates, so samples were further purified using the MoBio
140 PowerClean Pro kit (MoBio, Carlsbad, CA). DNA concentrations were assessed using a
141 Qubit 2.0 Fluorometer and dsDNA High Sensitivity assay (Life Technologies). Each sample
142 yielded a total of 30-50 ng DNA.

143

144 *SNP Analysis*

145 Genotyping-by-sequencing library preparation followed the protocols of Elshire et al.
146 (Elshire et al. 2011) with modifications. DNA extractions were first dried using a vacuum
147 centrifuge at 45°C, then resuspended in 15 µl dH₂O. To each sample, a uniquely barcoded
148 PstI adapter was added (2.25 ng per sample) (Morris et al. 2011). DNA digestion was
149 performed using 4U PstI-HF (New England Biolabs, Ipswich, MA) (Morris et al. 2011) in 1X
150 CutSmart BufferTM, with incubation at 37°C for two hours. Adapters were ligated with T4
151 DNA ligase in 1X ligation buffer (New England Biolabs, Ipswich, MA), followed by
152 incubation at 16°C for 90 min and 80°C for 30 min. Purification was performed using a
153 Qiagen MinElute PCR purification kit (Qiagen, Valencia, CA), with elution in 25 µl 1X TE.
154 PCRs were carried out in 50 µl volumes containing 10 µl purified DNA, 1X MyTaqTM HS
155 Master Mix (Bioline), and 1 µM each of PCR primers
156 5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCG
157 ATC*T and
158 5'CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCT
159 TCCGATC*T (where * indicates phosphorothioation). PCRs were run in an Eppendorf
160 Mastercycler Nexus under the following conditions: 72°C for 5 min, 95°C for 60 s, and 24
161 cycles of 95°C for 30 s, 65°C for 30 s, and 72°C for 30s, with a final extension step at 72°C
162 for 5 min. Library concentrations for each sample were assessed using a LabChip GXII
163 (Caliper Life Sciences) and all libraries were pooled (20 ng DNA per sample). Size
164 fractionation of the pooled library was achieved via electrophoresis on a 1.5% agarose gel,
165 with a 300 bp size range from 200 - 500 bp selected for sequencing. Sequencing was carried
166 out on one lane of an Illumina HiSeq 2500.

167

168 Reads were assessed for quality and trimmed for Illumina TruSeq2 adaptors using
169 Trimmomatic version 0.32 (Bolger et al. 2014). 112,510,564 paired end reads with
170 identifiable PstI adapters were sequenced. The sequencing protocol (similar to Dussex et al.
171 2015) employed a combinatorial barcode, which required matching partial-barcodes on both
172 ends of paired-end reads to identify the sample each came from. As the downstream TASSEL
173 version 3.0.167 (Bradbury et al. 2007) pipeline does not work with dual-ended barcodes,
174 unique sample-identifying barcodes (a concatenation of the partial barcodes on the R1 and R2
175 ends of each read) were replaced on both ends of the reads using custom Python scripts (see
176 Supporting Information) for subsequent processing via TASSEL UNEAK, with default
177 parameters, apart from: restriction enzyme (PstI), minimum number of tags required (5),

178 error-tolerance rate (0.03), minimum/maximum minor allele frequencies (MAF of 0.05 and
179 0.5), and minimum/maximum call rates (0 and 1). The UNEAK pipeline does not require a
180 reference genome as it uses network analysis and χ^2 tests to filter matched sequence tags to
181 remove most errors and paralogs (as described in detail by Lu et al. 2013). The pipeline was
182 developed for identifying SNPs from bi-allelic markers, and is thus well suited for use with
183 data from *Durvillaea*, which is diplontic (with a diploid macroscopic stage dominating the
184 life cycle: Thornber 2007).

185

186 The SNP dataset resulting from this pipeline had 75,712 sites, with heterozygous positions
187 represented by IUPAC ambiguity codes (e.g., the heterozygous position 'C/T' corresponds to
188 a 'Y' in the alignment). Due to the robustness of the binary distance metric (Jaccard index,
189 Hamers et al. 1989) used in downstream analyses, we were able to utilise all SNPs called by
190 UNEAK without the need to filter any out. Under-represented samples (with fewer than
191 10,000 reads assigned to them) were removed using custom scripts in R version 3.1.0 (R
192 Development Core Team 2013), leaving 73 samples (Table 1). As is common for GBS
193 datasets (Jarquin et al. 2014), the final filtered genotype matrix had a large amount (96.42%)
194 of missing data. R was also used for principle components analysis (PCoA): see supporting
195 information.

196

197 *Phylogenetic Analyses*

198 The sites in SNP alignments generated from random restriction enzyme digests, and aligned
199 in the absence of a reference genome, are undoubtedly evolving at a variety of rates (see
200 Introduction), and this variation should be taken into account in any phylogenetic estimation
201 efforts. The most common approach to account for rate heterogeneity in an alignment is to
202 model site-specific rates with a gamma distribution. However, alternative approaches,
203 whereby rates are free to vary without being constrained by a pre-specified distribution, have
204 been shown to out-perform the discrete gamma model (Lartillot and Philippe 2004; Pagel and
205 Meade 2004; Huelsenbeck and Suchard 2007), and these approaches may be particularly
206 well-suited to GBS-based SNP data. The phylogenetic software, IQ-TREE ver. 1.4.1 (Nguyen
207 et al. 2015) has an option that includes the FreeRate model (Soubrier et al. 2012) in its model
208 selection strategy, thus explicitly accounting for rate heterogeneity among sites in a pre-
209 specified distribution-free manner. Thus, we used this software package for all of our
210 phylogenetic analysis.

211

212 We first used IQ-TREE to identify the optimal model of evolution for the full dataset of
213 75,712 SNPs using the -m TESTNEWONLY+ASC option (where the '+ASC' flag is used to
214 account for ascertainment bias in SNP data). IQ-TREE was then executed in full mode to
215 infer phylogenetic trees under the maximum-likelihood (ML) criterion using the identified
216 evolutionary model. This was determined to be GTR+G4+ASC (where G4 refers to a gamma
217 distribution with four rate categories), with the following rate parameters: A-C: 0.875, A-G:
218 2.823, A-T: 0.809, C-G: 0.537, C-T: 2.895, G-T: 1.000; and base frequencies: A: 0.242, C:
219 0.253, G: 0.260, T: 0.246; a proportion of invariable sites of: 0.669, and a Gamma alpha
220 shape parameter of: 0.034. Ultra-fast bootstrap approximation (Minh et al. 2013) was used
221 with 10,000 bootstraps to assess node support, and the final tree was evaluated in FigTree
222 ver. 1.4.1 (Rambaut 2009) (Fig. 2). Note that, in line with other phylogenetic software, IQ-
223 TREE assigns the same likelihood to each base of an ambiguous site (including heterozygous
224 positions). This analysis was repeated independently ten times, to assess whether the
225 phylogenetic relationships inferred between *D. antarctica* and *D. chathamensis* were robust,
226 and particularly whether the SNP data supported monophyly for the *D. chathamensis* clade
227 that was indistinguishable from *D. antarctica* in previous analyses of mitochondrial and
228 nuclear loci (Fraser et al. 2010).

229

230 As well as including FreeRate models in our analysis to determine the optimal model of
231 evolution, we examined the effects of potential rate heterogeneity among sites by running an
232 additional analysis without the inclusion of a gamma rate distribution (i.e., with an
233 evolutionary model of GTR+ASC compared to GTR+G4+ASC, above). As above, we ran
234 the software in full mode with 10,000 bootstraps. Our aim was to determine the potential
235 effect of rate variation among sites on our final tree topology.

236

237 Next, we assessed how consistent the phylogenetic relationships estimated for *D. antarctica*
238 and *D. chathamensis* were, as a function of the number of SNPs used in the analysis. First,
239 we determined in IQ-TREE that the full dataset contained a total of 40,912 parsimony-
240 informative sites. We reduced the dataset to retain only these sites as, although including full
241 sequences can help to improve phylogenetic inference of branch lengths (Leaché et al. 2015),
242 we were primarily interested in tree topology for which only parsimony-informative sites are
243 needed. We used a series of bash commands to randomly extract sites, creating six SNP

244 datasets of lengths: 400, 1,000, 1,500, 2,000, 4,000, and 10,000, and retaining the original
245 dataset of 40,912 parsimony-informative sites (see Supporting Information for commands).
246 Ten random datasets were generated for each SNP length and each was then run through our
247 IQ-TREE pipeline, as outlined above. From each final phylogenetic tree estimate generated,
248 we extracted the bootstrap value for the node that first connected a *D. chathamensis*
249 individual to a sister *D. antarctica* individual (see Fig. 3). We also assessed whether the
250 overall phylogeny generated was consistent with the results from both the full dataset of
251 75,712 SNPs, and the full parsimony-informative dataset (40,912 SNPs), generating for each
252 replicate a binary decision (yes / no) concerning the monophyly of *D. chathamensis* as sister
253 to the *D. antarctica* Chatham Island/mainland New Zealand clades (see Results); i.e., whether
254 the reproductive isolation of *D. chathamensis* was supported.

255

256 In a final analysis, we assessed the distance between phylogenetic estimates obtained for the
257 various SNP datasets and the full parsimony-informative dataset (i.e., 40,912 informative
258 sites), using the `-rf_all` function in IQ-TREE to determine the Robinson-Foulds (RF) distance
259 between trees (Robinson and Foulds 1981). This metric measures the distance between
260 unrooted phylogenetic trees according to $(A + B)$, where A is the number of data partitions
261 implied by the first tree but not the second tree, and B is the number of data partitions implied
262 by the second tree but not the first tree. Rather than examining particular clades in isolation,
263 the RF metric takes all tree splits into account. We retrieved the range of RF distances within
264 each set of ten trees for the seven datasets, as well as the range of RF distances between the
265 six reduced-length SNP datasets and the full parsimony-informative dataset. In this way, we
266 determined the phylogenetic error associated with SNP choice; to determine the error
267 associated with phylogenetic construction, we performed a final test, taking one SNP file
268 from each of the ten variously-sized SNP datasets created previously, and running IQ-Tree on
269 that input file over ten replicates. We then calculated the RF distance between all ten trees
270 resulting from each same starting SNP file to assess phylogenetic error for each reduced-
271 length SNP dataset and for the full parsimony-informative dataset, as outlined above.

272

273 *Introgression*

274 As we are interested in assessing the genealogical basis for separate recognition of *D.*
275 *antarctica* and *D. chathamensis*, we performed introgression tests using the species
276 delimitation software SNAPP ver. 1.3.0 (Bryant et al. 2012) in BEAST ver. 2 (Bouckaert et

277 al. 2014). Specifically, we were interested in determining whether we could detect
278 introgression between Chatham Island populations of *D. antarctica* and *D. chathamensis* by
279 generating a species tree for all the samples in our dataset. We generated an input xml file
280 based on a binary file of the 75,712 SNPs (i.e., recoded with 012 coding), and used default
281 settings to run SNAPP. We ran the analysis for 10,000,000 MCMC generations and ensured
282 convergence of the resulting output log file using Tracer ver. 1.6
283 (<http://tree.bio.ed.ac.uk/software/tracer/>). We then visualised the posterior distribution of
284 species trees produced, using the DensiTree package associated with BEAST2, and looked
285 for evidence of introgression among taxa.

286

287 **Results**

288 Phylogenetic analyses based on the full dataset of 75,712 SNPs revealed four closely-related
289 but distinct genotypic assemblages, corresponding to (1) *D. chathamensis*; (2) Chatham
290 Island populations of *D. antarctica*; (3) mainland New Zealand populations of *D. antarctica*,
291 and (4) sub-Antarctic populations of *D. antarctica* (Fig. 2). PCoA analysis also supported
292 these geographic and phylogenetic clusters, with five PCs explaining 84.29% of the total
293 variation (PC1: 26.35%; PC2: 25.36%; PC3: 18.1%; PC4: 8.28%; PC5: 6.2%). Binary PCoA
294 clusters with PC1/PC2 and PC3/PC4 are shown in Fig. 4. Phylogenetic analyses using the full
295 dataset support the distinct phylogenetic status of the two Chatham Island morphotypes, with
296 consistent genome-wide differences between them across multiple sympatric localities. The
297 mainland New Zealand and Chatham Island populations of *D. antarctica* were resolved as
298 monophyletic, with *D. chathamensis* as a sister group within the *D. antarctica* complex (Fig.
299 2). These analyses also revealed strong spatial genetic differentiation, with distinct
300 geographic localities within each of the major groupings represented by distinct genotypic
301 clusters (Fig. 2), and with phylogeographic partitioning for both species among sites on
302 Chatham Island.

303

304 *Phylogenetic Uncertainty*

305 Our analysis of rate variation among sites (see Methods) showed there to be no difference –
306 other than minor differences in branch lengths – in topology from phylogenetic analyses with
307 and without the gamma distribution included in our evolutionary model (Fig. 2).

308

309 The results of our phylogenetic bootstrapping analyses among reduced-length datasets of
310 only parsimony-informative sites are presented in Fig. 3, where the bootstrap value
311 connecting *D. chathamensis* to its sister clade of *D. antarctica* can be seen for each of our
312 SNP datasets. In Figure 5, the number out of ten replicates for which the topologies returned
313 *D. chathamensis* as a monophyletic group is indicated. If we consider the full parsimony-
314 informative dataset (40,912 sites) to have provided the putatively most accurate phylogenetic
315 estimate (we feel this is reasonable, as the topology matches that from our full analysis of
316 75,712 sites, where monophyly of *D. chathamensis* is supported: Fig. 2), then these results
317 can be seen to reveal a large degree of uncertainty in bootstrap support for the node
318 connecting *D. chathamensis* to its sister *D. antarctica* clade for SNP datasets less than 10,000
319 characters in length (Fig. 3). However, even when bootstrap support at a given node is high,
320 obtaining a tree topology consistent with the full dataset (i.e. returning monophyly of *D.*
321 *chathamensis*) does not necessarily follow (Fig. 5). For example, although the mean bootstrap
322 support connecting *D. chathamensis* and *D. antarctica* exceeded 90% for datasets of $\geq 2,000$
323 SNPs, the topology only resolved *D. chathamensis* as monophyletic for 6/10, 6/10, and 7/10
324 replicates for SNP datasets of length 2,000, 4,000, and 10,000, respectively (Figs. 3 and 5).
325 Even within the shorter SNP datasets (e.g., $\leq 1,500$ SNPs), bootstrap support for the *D.*
326 *chathamensis* / *D. antarctica* relationship reached as high as 84% in individual replicates
327 when the topology was inconsistent (i.e., *D. chathamensis* was not monophyletic) with the
328 full dataset (both the 75,712 and the 40,912 SNP alignments).

329

330 RF distances for the reduced-length datasets reiterate the above findings, reflecting a high
331 degree of topological uncertainty with respect to choice of SNP number. For example, using
332 the full parsimony-informative dataset of 40,912 sites, RF distances (number of topological
333 differences between trees) ranged from 0-14, but the number of partitions disagreeing
334 between the trees generated with replicates of the 10,000 SNP datasets reached as high as
335 100, and for the 400-SNP datasets, reached as high as 138 (Table 2). As well as within-
336 dataset uncertainty, our analyses identified a high degree of between-dataset uncertainty. For
337 example, the shorter 10,000 SNP datasets resulted in trees that differed by up to 82 partitions
338 from those generated with the full parsimony-informative dataset (Table 2). In our final RF
339 tests examining error with respect to phylogenetic replication, we found that RF distances
340 were higher for phylogenetic trees produced from the same input SNP file when that input
341 file had a smaller number of SNPs. For example, the RF distance ranged from 8-80 for

342 replicated phylogenetics generated from a single 400-SNP input file, and from 0-14 for a
343 single 40,912-SNP input file (Table 2). As a result, a high degree of topological uncertainty
344 exists with respect to both the number of SNPs utilised in the phylogenetic analysis, and the
345 phylogenetic algorithm itself, although in each case, phylogenetic estimates become more
346 robust / similar as the number of parsimony-informative SNPs increased.

347

348 *Introgression*

349 Our SNAPP analysis resulted in a species tree that showed no support for introgression
350 between Chatham Island populations of *D. chathamensis* and *D. antarctica* (Fig. 6).

351

352 **Discussion**

353 *Non-targeted SNP data for species delimitation*

354 Our results confirm that SNP data from non-targeted approaches such as GBS have great
355 resolving-potential for phylogenetic analysis, including for the genealogical delimitation of
356 closely-related species. Using the full dataset (75,712 SNPs, of which 40,912 were
357 parsimony-informative), we obtained 100% consistent topology at nodes that separated *a*
358 *priori* taxonomic (*D. chathamensis* vs *D. antarctica*) and geographic groupings (for *D.*
359 *antarctica*: North Island New Zealand, South Island New Zealand, and the sub-Antarctic)
360 (Figs 2, 3, 5), implying that these phylogenetic estimates are robust. Under a variety of
361 species concepts (e.g. genealogical; biological; phylogenetic) (Donoghue 1985), our results
362 support the distinct species status of *D. chathamensis*, with strong support for the reciprocal
363 monophyly of *D. chathamensis* and its sister *D. antarctica* clade. Alongside our SNAPP
364 analysis, a lack of genetic intermediates argues against the possibility that their mtDNA and
365 chloroplast sequence similarity might reflect introgression, although evidence of
366 introgression from low levels of recent gene flow could be restricted to specific parts of the
367 genome, and older geneflow might be undetectable in our analyses. Detection of
368 introgression could also be limited by the inability of this approach to distinguish between
369 hemizygotes, where only one allele is sequenced for a particular SNP and individual, and
370 homozygotes (Davey et al. 2013). Further tests for introgression (Twyford and Ennos 2012;
371 Eaton et al. 2015) could be performed in future studies using a greater number of samples
372 from a greater number of populations and *Durvillaea* lineages. Alternatively, incomplete
373 lineage sorting could explain the shared alleles at cytoplasmic and nuclear loci. Genome-wide
374 SNP data have nonetheless allowed us to confirm the species status of *D. chathamensis*, when

375 previous multilocus DNA (Fraser et al. 2010), and morphological and ecological
376 phylogenetic analyses (Cheshire et al. 1995), had failed to clearly resolve them. These
377 findings highlight the utility of GBS data for resolving phylogenetic relationships among
378 closely-related species, and for detecting recent speciation events. Furthermore, the strong
379 divergences detected among these and other *Durvillaea* lineages in our analyses (*D.*
380 *antarctica* from mainland New Zealand, and from the sub-Antarctic) support previous
381 suggestions that *D. antarctica* may comprise several as-yet unrecognised species (Fraser et al.
382 2010).

383

384 Our results emphasize, however, the need to be cautious when analysing SNP data and
385 interpreting the resultant phylogenies. Results varied drastically depending on the number of
386 SNPs included in our reduced-length, parsimony-informative site analyses (Figs 3 and 5),
387 and these conflicting topologies often received high bootstrap support (Fig. 3). Substantial
388 phylogenetic conflict between replicate ML analyses was particularly apparent for datasets
389 with relatively few SNPs. Indeed, when analyses included 10,000 SNPs or fewer, *D.*
390 *chathamensis* was often resolved as paraphyletic with respect to its sister taxon *D. antarctica*
391 (NZ / Chatham).

392

393 GBS-generated SNP data are inherently patchy, with low coverage and high proportions of
394 missing data (Lu et al. 2013), and this kelp dataset was no exception, with the percentage of
395 missing data in the full dataset ranging from 90-99%, and from 71-99% in the reduced-length
396 datasets. Indeed, the process of removing problematic polysaccharides such as alginates
397 during kelp DNA extractions resulted in the amount of DNA used in our GBS library
398 preparation being low (30 - 50 ng per sample, compared to the 100 ng used by Elshire et al.
399 2011), which probably affected the number of reads obtained. Restriction enzyme digestion
400 can also vary in effectiveness due to factors such as base-composition heterogeneity among
401 taxa (Scaglione et al. 2012), influencing how much SNP data can be obtained via GBS or
402 RAD tag and making interspecific comparisons particularly prone to having larger amounts
403 of missing data. The coverage and depth of datasets will therefore vary for different taxa, as
404 will the number of SNPs needed to resolve phylogenies. A dataset with a smaller proportion
405 of missing data might be less likely to yield differing phylogenetic topologies when different
406 numbers of SNPs are included. Nonetheless, these results highlight the importance of using
407 as many SNPs as possible, and – importantly – of independently replicating phylogenetic

408 analyses to assess the robustness of the topology, rather than relying on bootstrap values
409 alone.
410
411 *SNP data as a tool to detect incipient speciation events: the case of Durvillaea*
412 Several evolutionary studies have indicated that repeated ecologically-driven transitions
413 (Rundle and Nosil 2005; Soria-Carrasco et al 2014) can generate rapid genetic divergence,
414 leading to multiple speciation events over short timeframes. Modelling studies have
415 suggested that reproductive isolation can potentially evolve within fewer than one hundred
416 generations (Hendry et al. 2007). In the case of *Durvillaea*, transitions from hollow-bladed
417 (buoyant) to solid-bladed (non-buoyant) morphology may be an important process driving
418 repeated and ongoing diversification. Other distinctive solid-bladed populations of *D.*
419 *antarctica* have been recorded at several localities across the Southern Hemisphere range of
420 this taxon, including South America (Ramírez and Santelices 1991) and the sub-antarctic
421 islands, for example Macquarie Island (Klemm and Hallam 1988), Marion Island and Gough
422 Island (Hay 1994). As in the case of *D. chathamensis* and *D. antarctica* on Chatham Island,
423 analysis of mtDNA from solid-bladed morphotypes from Gough, Marion and the Falkland
424 Islands has not shown any notable genetic differences between these and sympatric buoyant
425 plants (Fraser et al. 2010). Although a monophyletic origin for solid-bladed forms of
426 *Durvillaea* was originally proposed (Hay 1979a), both molecular (Fraser et al. 2010) and
427 morphological / ecological (Cheshire et al. 1995) cladistic analyses indicate that solid forms
428 have arisen multiple times in the genus. Genome-wide SNP data represent an ideal tool with
429 which to assess whether the multiple solid-bladed forms present in *D. antarctica* also
430 represent examples of incipient reproductive speciation. Broadly, it seems that parallel
431 divergence underpinned by repeated directional selection (e.g. Albertson et al. 2003; Protas et
432 al. 2006; Soria-Carrasco et al. 2014) represents a key force in driving predictable patterns of
433 biotic evolution.

434

435 *Sympatric speciation or repeated island invasions?*

436 Our analyses suggest that the Chatham Island lineages of *D. antarctica* and *D. chathamensis*
437 are not each other's closest relatives; instead, *D. antarctica* from Chatham Island and *D.*
438 *antarctica* from nearby mainland New Zealand appear to be monophyletic, with *D.*
439 *chathamensis* as a sister group within the broader *D. antarctica* complex (Fig. 2). Double
440 invasion (McPhail 1984) of Chatham Island by *Durvillaea* (rather than sympatric speciation)

441 thus seems the most likely explanation for this phylogenetic pattern. We propose that oceanic
442 dispersal followed by rapid ecomorphological divergence may explain the rapid evolution of
443 these sympatric congeners, emphasizing the likely role of dispersal and founder speciation in
444 driving diversification. Founder-blocking priority effects could explain the maintenance of
445 phylogeographic structure in highly-dispersive species such as *D. antarctica* (Fraser et al.
446 2009; Waters et al. 2013). Specifically, despite the vast numbers of *D. antarctica* plants
447 drifting at sea (Garden *et al.* 2014; Smith 2002), colonization events appear most likely to
448 occur when dispersing individuals reach shores that are unoccupied by conspecifics (Fraser et
449 al. 2009; Waters et al. 2013). These data also add to the wealth of evidence for recent
450 colonization of the Chatham Islands from mainland source populations, followed by founder
451 speciation (Trewick 2000; Paterson et al. 2006; Goldberg et al. 2008; Heenan et al. 2010;
452 Goldberg and Trewick 2011).

453

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465

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644

645 **Data accessibility:** the SNP data set and dataset generation commands are provided as
646 supporting information.

647

648 **Author contributions:** CIF, AM and JMW designed the study; CIF did the laboratory
649 analysis; CIF wrote the first draft; AM and AC analyzed the data. All authors had input on
650 writing the paper and gave final approval for publication.

651

652 **TABLES**

653 **Table 1:** Number of *Durvillaea* samples per site used in downstream phylogenetic analyses
 654 (total: 73)

655

656	Clade	Site / region	# Samples
657	<i>D. antarctica</i> sub-Antarctic	Falkland Islands	7
658		Marion Island	3
659	<i>D. antarctica</i> New Zealand mainland	Banks Peninsula	5
660		Raramai Tunnels	1
661		Cape Campbell	4
662		Wellington	4
663		Maori Bay	2
664	<i>D. antarctica</i> Chatham Island	Wharekauri	5
665		Whangamoe Inlet	8
666		Waitangi West	9
667	<i>D. chathamensis</i> Chatham Island	Wharekauri	9
668		Whangamoe Inlet	7
669		Waitangi West	9

Table 2: A measure of the range of Robinson-Foulds distances among groups of ten trees estimated for random subsets of parsimony-informative SNPs (SNP choice (within), among the ten trees estimated for each listed SNP subset and the ten trees generated with the full parsimony-informative (40,912 sites) dataset (SNP choice (across)); and among sets of ten trees derived from the same starting input SNP file (phylogenetic error).

No. of SNPs in dataset	SNP choice (within)	SNP choice (across)	Phylogenetic error
400	118-138	110-130	8-80
1,000	106-132	96-122	0-36
1,500	88-124	74-114	0-40
2,000	96-124	74-104	0-58
4,000	80-110	66-94	0-38
10,000	64-100	56-82	0-58
40,912	0-14	-	0-14

Figure legends

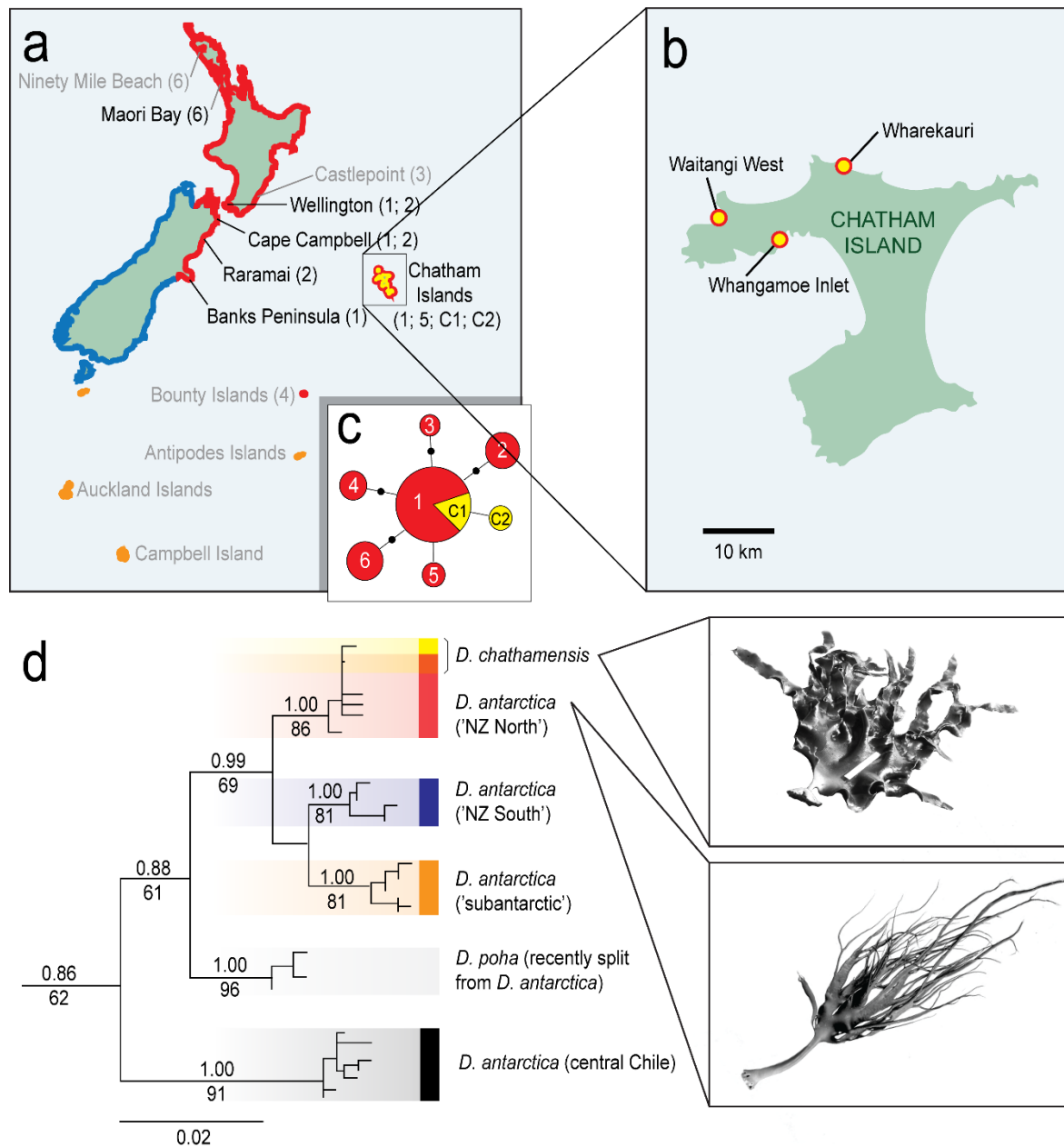


Figure 1: Distribution, phylogeny, and sampling of *Durvillaea antarctica* and *D. chathamensis*. a) distributions of lineages, with colors corresponding to those in other panels. *cox1* haplotypes for each site sampled from the *D. chathamensis* / *D. antarctica* ‘NZ North’ clade (Fraser et al. 2010) are indicated in parentheses after site names, and sites from which samples were used in this study are shown in black text. b) Sampling sites for sympatric *D. antarctica* and *D. chathamensis* used in this study. c) mtDNA (*cox1*) haplotype network for the *D. chathamensis* / *D. antarctica* ‘NZ North’ clade (for all samples used in Fraser et al. 2010). d) mtDNA (*cox1*) phylogeny of the *D. antarctica* / *D. chathamensis* / *D. poha* clade

(from Fraser et al. 2010). Photographs illustrate the morphological differences between *D. antarctica* and *D. chathamensis*.

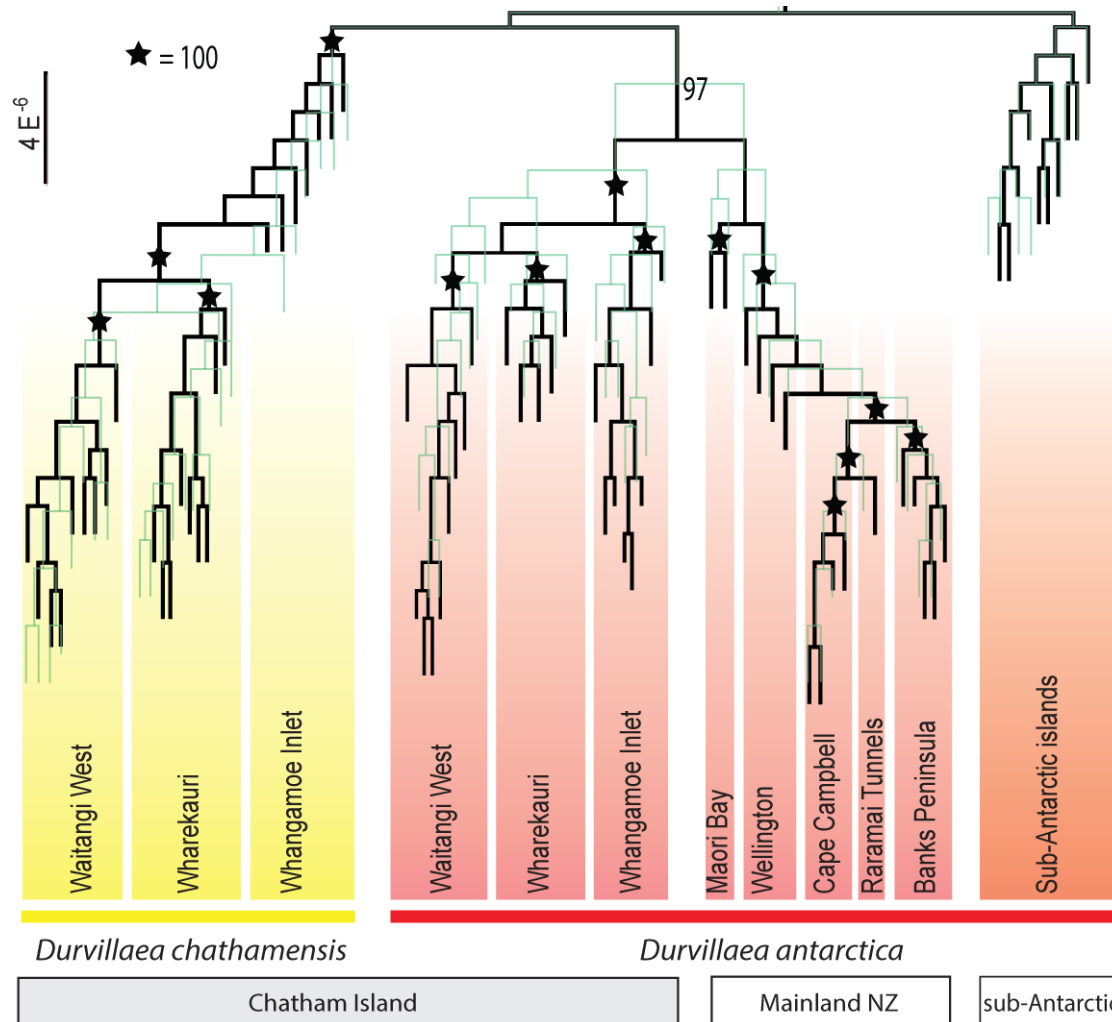


Figure 2: Maximum Likelihood tree for Chatham Island and mainland NZ *Durvillaea* populations based on the full dataset of 75,712 SNPs (black lines). Node support (10,000 bootstrap replicates) is shown for major branches. ML tree for a second analysis using an evolutionary model without gamma rate variation is shown by underlying thin green lines.

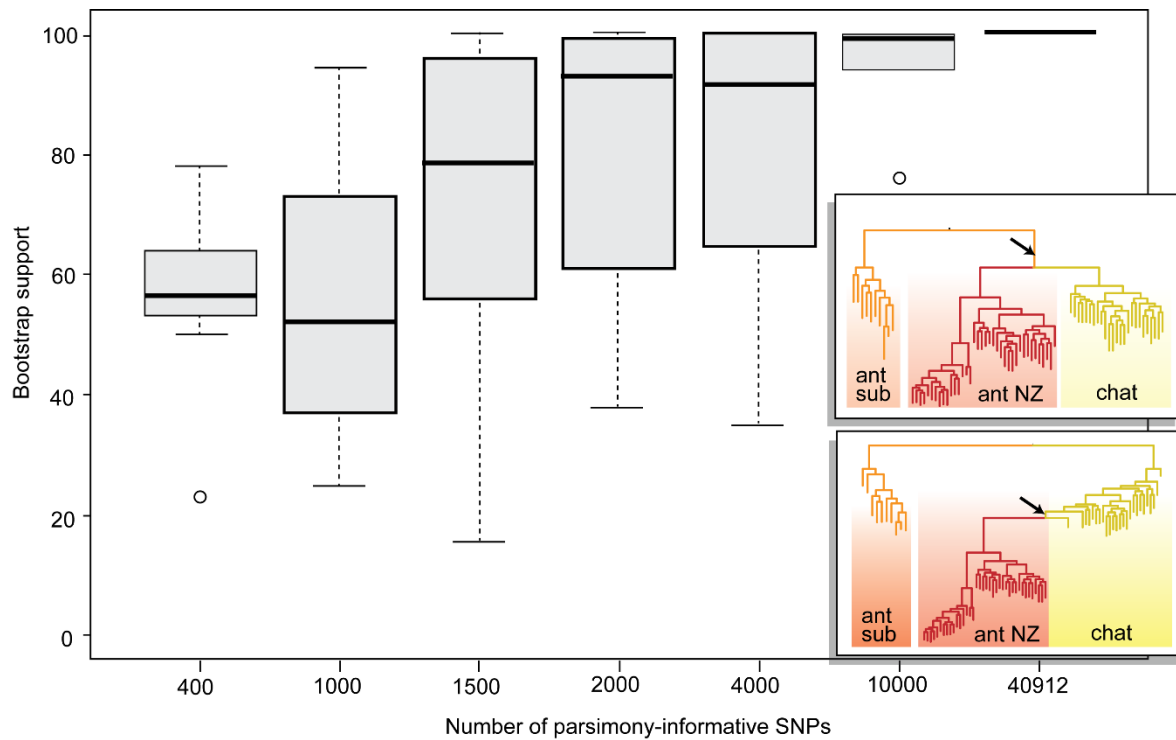


Figure 3: Bootstrap support as a function of number of SNPs used in analyses. Boxplots indicate the range of bootstrap support at the node connecting *D. chathamensis* to its sister clade (*D. antarctica* NZ/Chatham) for ten independent replicate analyses using randomly selected, parsimony-informative subsets of the data (400, 1,000, 1,500, 2,000, 4,000, 10,000 SNPs) and the full parsimony-informative dataset (40,912 SNPs). Inset: examples to demonstrate the location of the node (marked with an arrow) connecting *D. chathamensis* ('chat') to its sister *D. antarctica* clade ('ant NZ,' from the New Zealand mainland and Chatham Island; the sub-Antarctic clade is labelled 'ant sub' in the case of a phylogenetic tree where the monophyly of *D. chathamensis* is supported (upper inset) and not supported (lower inset).

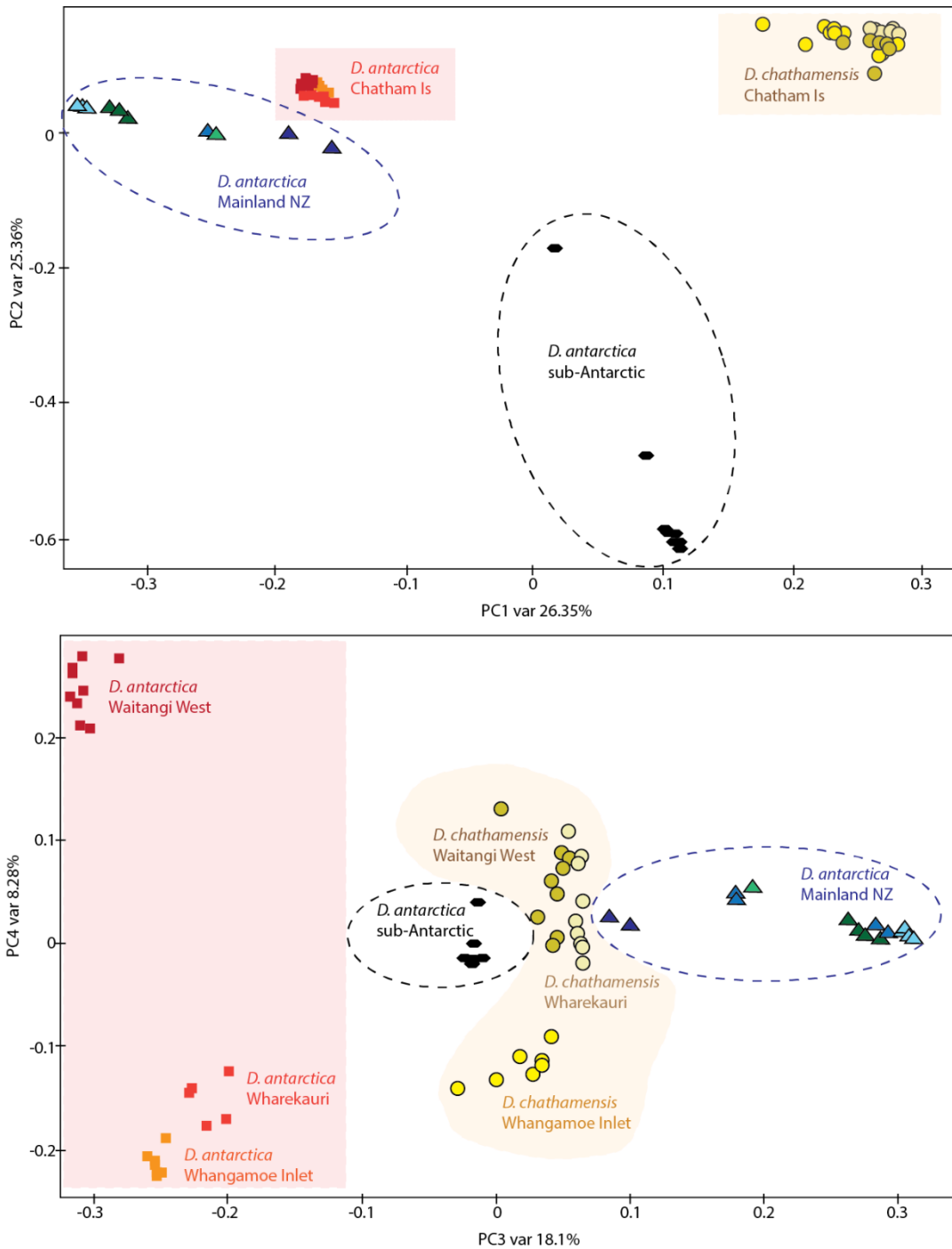


Figure 4: PCoA plots showing geographic and phylogenetic clusters. The regions occupied by the two Chatham Island groups (*D. antarctica* and *D. chathamensis*) are indicated by red and orange shading, with individuals shown as square and circular symbols, respectively. The regions occupied by the two outgroups, *D. antarctica* from the New Zealand mainland and from the sub-Antarctic, are circled, with individuals indicated by triangular and hexagonal symbols, respectively.

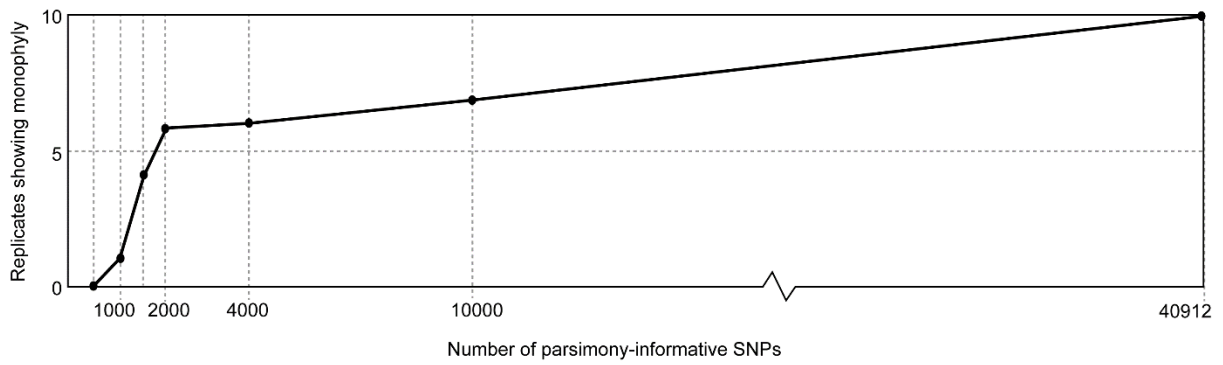


Figure 5: The number out of each set of ten replicates which displayed a final tree topology where *D. chathamensis* was recovered as a monophyletic group, sister to the Chatham and New Zealand mainland *D. antarctica* clades, consistent with the topology resolved from the full dataset (i.e. both the 75,712 full, and the 40,912 parsimony-informative, alignments).

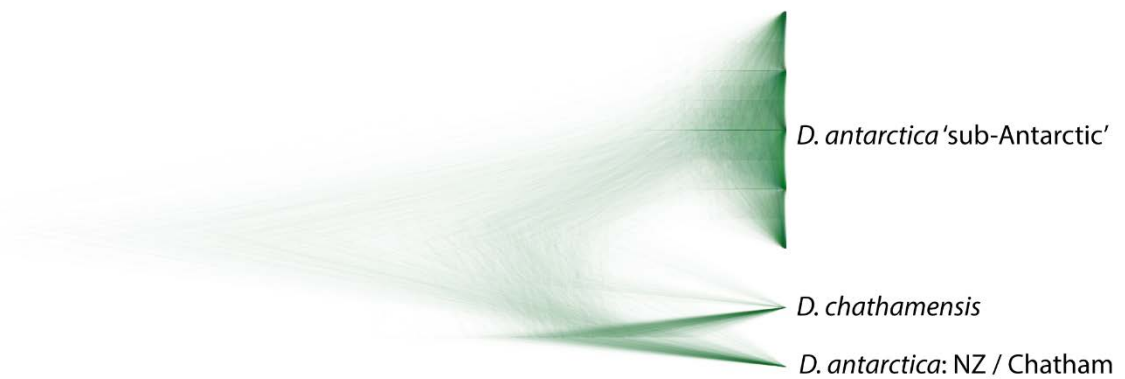


Figure 6: Species delimitation analysis (DensiTree), showing no evidence for introgression between *D. chathamensis* and Chatham Island *D. antarctica* clades.