Received: 30 September 2019 | Revised: 25 February 2020 | Accepted: 27 February 2020

DOI: 10.1111/jbi.13843

RESEARCH PAPER

Journal of Biogeography | WILEY

Multiple late-Pleistocene colonisation events of the Antarctic pearlwort *Colobanthus quitensis* (Caryophyllaceae) reveal the recent arrival of native Antarctic vascular flora

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Abstract

Aim: Antarctica's remote and extreme terrestrial environments are inhabited by only two species of native vascular plants. We assessed genetic connectivity amongst Antarctic and South American populations of one of these species, *Colobanthus quitensis*, to determine its origin and age in Antarctica.

Location: Maritime Antarctic, sub-Antarctic islands, South America.

Taxon: Antarctic pearlwort *Colobanthus quitensis* (Caryophyllaceae).

Methods: Four chloroplast markers and one nuclear marker were sequenced from 270 samples from a latitudinal transect spanning 21–68° S. Phylogeographic, population genetic and molecular dating analyses were used to assess the demographic history of *C. quitensis* and the age of the species in Antarctica.

Results: Maritime Antarctic populations consisted of two different haplotype clusters, occupying the northern and southern Maritime Antarctic. Molecular dating analyses suggested *C. quitensis* to be a young (<1 Ma) species, with contemporary population structure derived since the late-Pleistocene.

Main conclusions: The Maritime Antarctic populations likely derived from two independent, late-Pleistocene dispersal events. Both clusters shared haplotypes with sub-Antarctic South Georgia, suggesting higher connectivity across the Southern Ocean than previously thought. The overall findings of multiple colonization events by a vascular plant species to Antarctica, and the recent timing of these events, are of significance with respect to future colonizations of the Antarctic Peninsula by vascular plants, particularly with predicted increases in ice-free land in this area. This study fills a significant gap in our knowledge of the age of the contemporary Antarctic

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Funding information
Spanish Ministry of Economy and Competitiveness, Grant/Award Number: ALIENANT CTM2013-47381-P; NERC, Grant/Award Number: NE/P003079/1; Instituto Antártico Chileno, Grant/Award Number: RG_02-13 and RT_11-13; British Antarctic Survey, Carlsberg Foundation Grant number CF18-0267

Handling Editor: Jim Provan

terrestrial biota. Adding to previous inferences on the other Antarctic vascular plant species (the grass *Deschampsia antarctica*), we suggest that both angiosperm species are likely to have arrived on a recent (late-Pleistocene) time-scale. While most major groups of Antarctic terrestrial biota include examples of much longer-term Antarctic persistence, the vascular flora stands out as the first identified terrestrial group that appears to be of recent origin.

KEYWORDS
angiosperm, Antarctica, biogeography, dispersal, island, pearlwort, South America, Southern Ocean

1 | INTRODUCTION

Antarctic terrestrial ecosystems experience some of the most extreme conditions on Earth. Estimates of current ice-free land surface area range from ~0.2% to 0.4% (Burton-Johnson, Black, Fretwell, & Kaluza-Gilbert, 2016; Terauds et al., 2012), with glacial models suggesting that most if not all of this area has been covered by ice during multiple glacial cycles (DeConto & Pollard, 2016; Pollard & DeConto, 2009). Significant ice sheet expansions occurred in the Miocene (23–5 Ma), Pliocene (5–2.6 Ma) and Pleistocene (2.6 Ma–10 ka), culminating in the Last Glacial Maximum (LGM), *c*. 33–14 ka. During the climatic extreme of the LGM, most of the continent's fringing ice shelves are thought to have extended to the edge of the continental shelf, apparently leaving little possibility for survival of terrestrial life on the continent. This has led to a widely held view that most of the contemporary Antarctic biota must be of recent (post-LGM) origin (Convey et al., 2008).

Recent biological research has challenged this view, revealing many examples of species with long-term pre-glacial persistence. Examples can be found within most major groups of Antarctic extant terrestrial biota (e.g. invertebrates, lichens, mosses, diatoms and microbial groups; Allegrucci, Carchini, Todisco, Convey, & Sbordoni, 2006; Bennett, Hogg, Adams, & Hebert, 2016; Biersma et al., 2017; Biersma, Jackson, Stech, et al., 2018; Chong, Pearce, & Convey, 2015; Convey et al., 2008, 2009; Convey & Stevens, 2007; De Wever et al., 2009; Iakovenko et al., 2015; Pisa et al., 2014; Vyverman et al., 2010), extending far back in time from hundreds of thousands to multi-million-year time-scales. While this evidence has led to a paradigm shift in the perception of the age of Antarctic life, at present, biological and glaciological evidence still does not align and continues to challenge our understanding of the glacial history of Antarctica (Convey et al., 2008; Convey & Stevens, 2007).

The flora of Antarctica has a low species richness, and includes just *c*. 112 species of mosses (Ochyra, Smith, & Bednarek-Ochyra, 2008), *c*. 27 species of liverworts (Bednarek-Ochyra, Vana, Ochyra, & Smith, 2000) and two species of native angiosperms, the Antarctic pearlwort *Colobanthus quitensis* (Kunth.) Bartl. (Caryophyllaceae) and the Antarctic hair grass *Deschampsia antarctica* Desv. (Poaceae). Recent molecular research has revealed the Antarctic bryophyte flora to comprise a mixture of long-term survivors (Biersma et al., 2017; Biersma, Jackson, Stech, et al., 2018; Ochyra, 2003; Pisa et al., 2014) and more recent arrivals (Biersma, Jackson, Bracegirdle, et al., 2018; Biersma et al., 2017; Kato, Arikawa, Imura, & Kanda, 2013). While long-term survivors can be found within the bryoflora, the low diversity within the vascular flora suggests that it may be of recent origin. Fasanella, Premoli, Urdampilleta, González, and Chiapella (2017), studying the genetic diversity within *D. antarctica*, recently detected 17 nuclear DNA and six plastid DNA haplotypes in Patagonia, while Antarctica had just one nuclear and four plastid DNA haplotypes. As the haplotypes present in Antarctica were only a small fraction of those present in Patagonia, and the nuclear haplotype in Antarctica was also found in Patagonia, this suggested that the species likely dispersed to the Antarctic in the mid- to late-Pleistocene.

Although the genetic diversity of *C. quitensis* has previously been studied (Acuña-Rodríguez, Oses, Cortés-Vasquez, Torres-Díaz, & Molina-Montenegro, 2014; Androsiuk, Chwedorzewska, Szandar, & Giełwanowska, 2015; Cuba-Díaz, Cerda, Rivera, & Gómez, 2017; Cuba-Díaz, Klagges, et al., 2017; Gianoli et al., 2004; Koc et al., 2018; Lee & Postle, 1975; Parnikoza, Maidanuk, & Kozeretska, 2007), as yet, no clear conclusions can be drawn about the age of the species in Antarctica (Parnikoza, Kozeretska, & Kunakh, 2011). This is mainly due to logistical and technical constraints, such as restricted geographical sampling and small sample sizes, and the genetic markers used being unsuitable for molecular dating techniques. Studies with more thorough sampling across the species' biogeographic range and the use of more appropriate, DNA sequence-based markers are hence required to assess the timing of divergence among populations on either side of the Southern Ocean.

Here, by applying population genetic and molecular dating analyses to *C. quitensis* specimens collected from across the widest range of localities sampled to date, we aimed to assess (a) whether *C. quitensis* may have survived the LGM in refugia in the Maritime Antarctic (encompassing the Antarctic Peninsula, South Shetland Islands and South Orkney Islands), or (b) whether its arrival in these regions is a more recent post-glacial event.

2 | MATERIALS AND METHODS

2.1 | Sampling

The full biogeographic range of *C. quitensis* includes areas of the Antarctic Peninsula north from 69° S (Convey, Hopkins, Roberts, & Tyler, 2011), the South Shetland Islands, the South Orkney Islands, South Georgia, the Falkland Islands, the southern ranges of Chile and Argentina, the High Andes regions of Chile, Argentina, Ecuador and Bolivia, and extends into Mexico (Moore, 1970). Our dataset consisted of 270 samples collected from across the southern part of the species' biogeographic range, where it is most commonly found (see Figure 1a; for the full distribution of the species see Figure 1b). To allow for a detailed study of both within-population and wider geographical variation, we combined two types of available datasets: (a) a population level dataset of a total of 200 freshly collected samples from 19 different field locations, with several (n > 1) samples collected per location, and (b) a dataset derived from single samples (n = 1) from 70 locations, derived from herbarium specimens, single fresh collections and one previously sequenced specimen from GenBank (see Table S1, Appendix S1). We included as many samples from the two datasets as possible in all analyses.

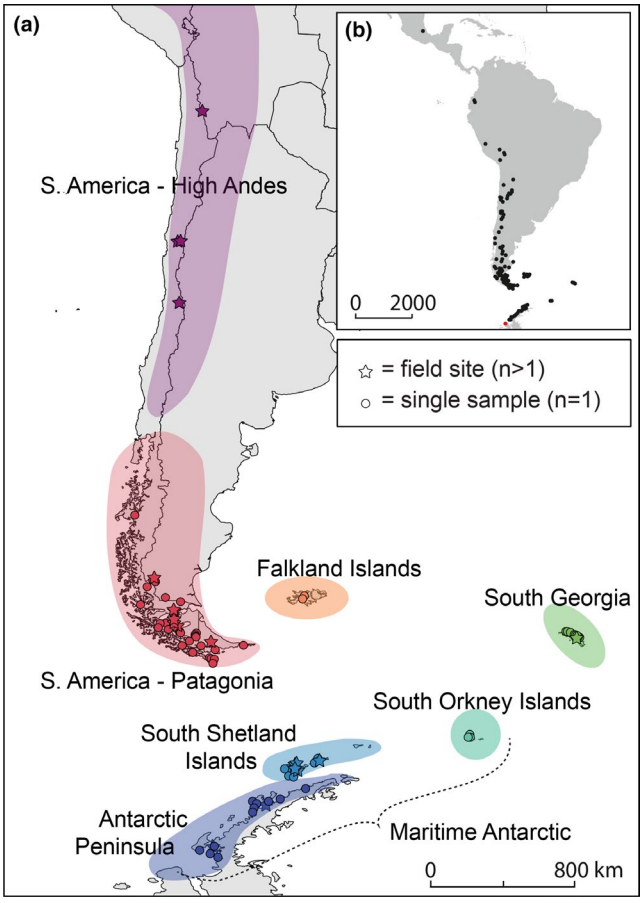

FIGURE 1 (a) Map with sampling locations of *Colobanthus quitensis*, comprising locations of freshly collected samples from field sites (stars; with multiple samples per site) and samples from herbaria (circles; representing a single sample per location). Biogeographical regions are indicated with different colours. (b) Global distribution of *C. quitensis* (black dots representing the distribution of *C. quitensis*, following Moore, 1970; red dot representing southern limit of *C. quitensis*, following Convey et al., 2011)

For the phylogenetic analyses, we included 21 samples (four from GenBank and 17 newly sequenced samples) from seven additional *Colobanthus* species as outgroups, viz., *C. subulatus* (D'Urv.) Hook.f., *C. kerguelensis* Hook.f., *C. apetalus* (Labill.) Druce, *C. strictus* Cheesem., *C. hookeri* Cheesem., *C. affinis* (Hook.) Hook.f. and *C. masonae* L.B.Moore. For the molecular dating analyses, nine specimens from *Sagina* species (seven from GenBank and two newly sequenced samples) were included as outgroups, based on the relationships reported by Dillenberger and Kadereit (2014) and Greenberg and Donoghue (2011) (see Table S1 for information on samples and GenBank accession numbers). Herbarium samples were obtained from the herbaria of the British Antarctic Survey, UK, and the University of Magallanes in Punta Arenas, Chile (herbarium codes AAS and HIP, respectively).

2.2 | DNA extraction, PCR amplification, sequencing and alignment

The DNA regions selected for comparison included one nuclear ribosomal (nrDNA) marker, the ribosomal Internal Transcribed Spacer (*ITS*) region (*ITS*1-5.8S-*ITS*2) and four chloroplast (cpDNA) markers, viz., the *ndhF-rpl32* spacer, *rpl32-trnL* spacer, *trnQ-rps16* spacer and the *atpB-rbcL* spacer. For the first dataset (a), comprising only field-fresh collections, *ndhF-rpl32R*, *rpl32F-trnL* and *ITS* were sequenced, while for the second dataset (b), all markers were sequenced (see Table S1 for sampling locations and sequence details).

DNA was extracted from leaf tissue using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), and E.Z.N.A. Plant DNA Kit (Omega Biotek, USA) following the manufacturers' instructions, using liquid nitrogen and a mortar and pestle for tissue disruption. PCR amplification was carried out using the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany) and Platinum Taq DNA polymerase (Invitrogen, Life Technologies) according to the manufacturers' instructions, with the addition of 1 μl of bovine serum albumin. Primer information and annealing temperatures are given in Table S2 (Appendix S1). Forward and reverse sequencing was performed by LGC Genomics (Berlin, Germany) and Macrogen (Seoul, Korea).

Forward and reverse sequences were combined and aligned with PRANK 140603 (Löytynoja & Goldman, 2008), using default settings, with minor corrections made manually. Models of DNA sequence evolution were selected using JMODELTEST 2.1.10 (Darriba, Taboada, Doallo, & Posada, 2012; Guindon & Gascuel, 2003) implementing the SPR base tree search, G rate variation option and the corrected Akaike information criterion (AICc) method for model comparisons. This found the most appropriate models were JC for *ITS*, TPM1uf for *atpB-rbcL*, and TPM1uf+G for *ndhF-rpl32R*, *rpl32F-trnL* and *trnQ-rps16*.

2.3 | Phylogenetic and population genetic analyses

Bayesian analyses were performed using MRBAYES 3.2 (Ronquist et al., 2012). All analyses were run for 25×10^6 generations, applying default settings and the closest match to the JMODELTEST identified substitution models per partition (cpDNA: nst = 6, rates = gamma; *ITS*: nst = 1, rates = equal), sampling every 1.0×10^3 generations, and omitting the first 25% of trees as burn-in. Convergence was assessed using TRACER 1.6 (Rambaut, Suchard, Xie, & Drummond, 2014) by verifying that split frequencies had an average standard deviation of <0.01 and all posterior parameter estimates exceeded effective sample sizes by >200. Maximum clade credibility trees with median heights were visualized using FIGTREE 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Maximum likelihood analyses were performed using RAxML-GUI 1.3.1 (Silvestro & Michalak, 2012), applying the 'bootstrap+consensus' option (1,000 iterations) using the JMODELTEST identified models of evolution with other settings as default. We inferred trees for *ITS*, *trnQ-rps16*, *atpB-rbcL* and the combined chloroplast regions *ndhF-rpl32R* and *rpl32F-trnL* (the latter were combined because they were always present together), as well as generating a combined tree using *ndhF-rpl32R*, *rpl32F-trnL* and *ITS* (for which data from most specimens were included; *n* = 232). To assess for topological incongruence among phylogenies derived from the cpDNA and nrDNA partitions, we used >70% bootstrap (BS) and >95% posterior probability support (PP) thresholds. Topological conflicts were assumed to be significant if two conflicting relationships for the same set of taxa were both supported with bootstrap values ≥70% and PP ≥95%. Phylogenetic analyses of the combined cpDNA and nrDNA datasets were conducted using an alignment containing unique sequences only (for a list of unique sequences see Table S1), that were extracted from the full dataset using GENEIOUS 9.1.8 (https://www.geneious.com).

To assess within-species variation in *C. quitensis* according to biogeographic region, TCS phylogenetic networks (Templeton, Crandall, & Sing, 1992) were built using PopART (Leigh & Bryant, 2015), with default settings. Networks were made for each marker separately (*ITS*, *trnQ-rps16*, *atpB-rbcL* and the combined chloroplast regions *ndhF-rpl32R* and *rpl32F-trnL*) and from a combined dataset containing *ndhF-rpl32R*, *rpl32F-trnL* and *ITS* sequences. We calculated standard genetic diversity indices for all markers in ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2010). We additionally carried out Tajima's *D* (Tajima, 1989) and Fu's F_s (Fu, 1997) neutrality tests for the cpDNA regions only. For the combined *ndhF-rpl32R* and *rpl32F-trnL* dataset, we also calculated molecular diversity indices for biogeographic regions with sample sizes of >10 (High Andes, Patagonia, South Georgia, South Shetland Islands and Antarctic Peninsula). Additionally, pairwise F_{ST} and Φ_{ST} (Excoffier, Smouse, & Quattro, 1992) values (using Kimura 2P genetic distances; Kimura, 1980) were calculated between these biogeographic regions, with 10,000 dataset permutations to assess significance. Numbers of variable and parsimony informative (PI) sites were calculated using MEGA7 (Kumar, Stecher, & Tamura, 2016).

2.4 | Molecular dating

Relative divergence times and ages for *C. quitensis* were calculated using STARBEAST 2.5.1 (Bouckaert et al., 2014) on the combined *ndhF-rpl32R*, *rpl32F-trnL* and *ITS* dataset that also included nine *Sagina* specimens as outgroups. Analyses were performed using the unique haplotypes only. As there are no fossil or geological calibration points available for molecular dating within the genus, we used two alternative methods for date calibration: (a) employing a previously calculated divergence date for the split between *Colobanthus* and *Sagina* from Dillenberger and Kadereit (2017), in the form of a lognormal prior of 3.44 Ma and a 95% highest posterior density interval of 1.34–5.91 Ma, and (b) applying a substitution rate on the cpDNA partition of $0.8 \pm 0.06 \times 10^{-9}$ subst./site/year, based on the rate estimated for chloroplast noncoding regions by Yamane, Yano, and Kawahara (2006) and previously applied on the Caryophyllaceae species *Silene acaulis* (Gussarova et al., 2015). For both methods, we applied a coalescent Bayesian Skyline tree prior, strict molecular clock and the JC69 and GTR+G models of evolution for *ITS* and cpDNA markers, respectively, allowing independent clocks for both genomic partitions. We used a linear multi-species coalescent with constant root as well as the appropriate ploidy level gene trees for both partitions. All runs had a chain length of 1.0×10^8 generations, logging parameters every 5.0×10^3 generations. Convergence was assessed in Tracer as described above. A maximum clade credibility tree with median node heights and 10% burn-in was constructed using TREEANNOTATOR 2.5.1 (Bouckaert et al., 2014) and visualized using FIGTREE 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

3 | RESULTS

3.1 | Genetic diversity

Of all markers, the combined *rpl32-trnL* and *ndhF-rpl32* regions were the most variable, followed by *ITS*, *atpB-rbcL* and *trnQ-rps16* (containing 16, 7, 5 and 3 PI sites, respectively; Table 1). Amongst the different biogeographic regions, Patagonia showed the highest molecular diversity (gene diversity, variable and parsimony informative sites and nucleotide diversity), followed by the High Andes, the Antarctic Peninsula and South Georgia, with no variation being detected within the South Shetland Islands (Table 1). The neutrality tests revealed a significant negative Fu's F_s value for the combined *atpB-rbcL* and the combined *ndhF-rpl32* and *trnQ-rps16* datasets, indicating a likely recent population expansion or change in selection for these DNA regions within the species.

3.2 | Phylogenetic and population genetic analyses

The phylogenetic analyses revealed no significant topological incongruences among *rpl32-trnL* and *ndhF-rpl32* and *ITS* partitions, allowing for a combined analysis of data from these cpDNA and nrDNA

TABLE 1 (a) Summary of genetic diversity indices on all markers within *Colobanthus quitensis*. Tajima's *D* and Fu's F_s neutrality tests were performed on chloroplast markers (monophyletic groups only). (b) Genetic indices of biogeographic regions with sample sizes of $n > 10$ within the concatenated *ndhF-rpl32* and *rpl32-trnL* dataset

Marker	*n*	bp[a]	v	PI	π	*h*	Tajima's *D* (*p*)	Fu's F_s (*p*)
(a)								
ITS	263	536	8	7	0.005 ± 0.003	0.771 ± 0.025	N.A.	N.A.
trnQ-rps16	79	712	6	3	0.003 ± 0.002	0.604 ± 0.076	−1.059 (.147)	−1.495 (.279)
atpB-rbcL	66	822	8	5	0.002 ± 0.001	0.941 ± 0.033	−1.864 (.006)*	−6.627 (.001)*
ndhF-rpl32+rpl32-trnL	226	979	28	16	0.009 ± 0.004	0.837 ± 0.023	−1.552 (.030)*	−3.494 (.251)
(b)								
High Andes	52	923	6	5	0.007 ± 0.004	0.799 ± 0.023	N.A.	N.A.
Patagonia	75	968	22	11	0.004 ± 0.002	0.915 ± 0.016	N.A.	N.A.
South Georgia	16	913	2	1	0.001 ± 0.001	0.617 ± 0.135	N.A.	N.A.
S. Shetland Is.	89	913	0	0	0.000 ± 0.000	0.044 ± 0.030	N.A.	N.A.
Ant. Peninsula	29	913	2	2	0.001 ± 0.001	0.687 ± 0.050	N.A.	N.A.

Abbreviations: bp[a]: no. of usable base pairs (loci < 5.0% missing data); For Tajima's *D* and Fu's F_s neutrality tests $p < .05$ is significant (*); *h*: gene diversity; *n*: number of samples; PI: parsimony informative sites; v: variable sites; π: nucleotide diversity (average over locus).

regions (Figure 2; see Figure S1.1 and S1.2a–d in Appendix S1 for phylogenetic trees of combined and single markers, respectively). Phylogenetic analyses of *C. quitensis* indicated a north-to-south expansion, with an early split of several High Andes populations from the remaining biogeographic regions (Figure 2). Antarctic haplotypes were associated with two different clades: one clade contained specimens from the northern Maritime Antarctic, South Shetland Islands and South Georgia, while the other clade consisted of a polytomy containing specimens from the southern Antarctic Peninsula as well as many other biogeographic regions. Other clades contained High Andes samples from the southernmost Andes location (La Parva; see Table S1), and Patagonian specimens, respectively.

For the population genetic analyses of *ITS*, the 263 individuals analysed yielded nine unique haplotypes, while for the combined *rpl32-trnL+ndhF-rpl32* regions, the 226 individuals analysed yielded 28 haplotypes. The 79 and 66 individuals analysed for *trnQ-rsp16* and *atpB-rbcL* both resulted in seven haplotypes. The TCS networks of *ITS* (Figure 3a) and *rpl32-trnL+ndhF-rpl32* (Figure 3b) both revealed that the distribution of Antarctic specimens fell among different groups: in the *ITS* network, one of these consisted solely of specimens from the northern Maritime Antarctic, South Shetland Islands and South Georgia, while in the *rpl32-trnL+ndhF-rpl32* network, most of these specimens fell within a common sequence haplotype shared with South American specimens. Conversely, specimens from the southern Maritime Antarctic fell within a group shared with South American specimens within the *ITS* network, while these specimens formed distinct haplotypes in the *rpl32-trnL+ndhF-rpl32* network. The combined analysis of these markers (Figure 3c) revealed that specimens from the Maritime Antarctic fell into two distinct groupings: one containing specimens from the southern Antarctic Peninsula and the other containing specimens from the South Shetland Islands plus northern Antarctic Peninsula (Figure 3d). Both groupings also contained one or several specimens from South Georgia, respectively. The *trnQ-rsp16* network (Figure 3e) also showed two distinct haplotypes containing Antarctic specimens, however both were shared with South American specimens. The *atpB-rbcL* network (Figure 3f) showed only one haplotype containing Antarctic specimens, which was shared with South American specimens.

All pairwise F_{ST} and Φ_{ST} comparisons between biogeographic regions were significant (Table 2), with the South Shetland Islands showing particularly high population differentiation in haplotypic diversity (F_{ST}), followed by South Georgia and the Antarctic Peninsula. Taking into account molecular distances (Φ_{ST}), the High Andes populations were particularly differentiated from most other regions.

3.3 | Divergence time analysis

The estimated age using the fossil-calibrated method (I) revealed an earlier split of the most recent common ancestor (T_{MRCA}; split *Sagina* – *Colobanthus*) than the rate-informed dating analysis (II) (Table 3). Both methods suggested that the genus *Colobanthus* diverged throughout the course of the Pleistocene, and that *C. quitensis* originated *c.* 0.181 (0.042–0.431) to 0.666 (0.401–0.999) Ma (method I and II, respectively; i.e. the calculated age of the root of *C. quitensis*, corresponding to the split between the majority of the High Andes populations and the remaining populations). Further date estimates for divergences of other populations within this young species were not possible due to a lack of sufficient variation within the sequenced DNA regions. Rates for both partitions calculated using method (I) were $7.20 \pm 0.06 \times 10^{-9}$ subst./site/year for *ITS*, and $2.27 \pm 0.06 \times 10^{-8}$ subst./site/year for *ndhF-rpl32R+rpl32F-trnL*. The rate calculated for *ITS* using method (II) was approximately sixfold lower, at $1.13 \pm 0.01 \times 10^{-9}$ subst./site/year.

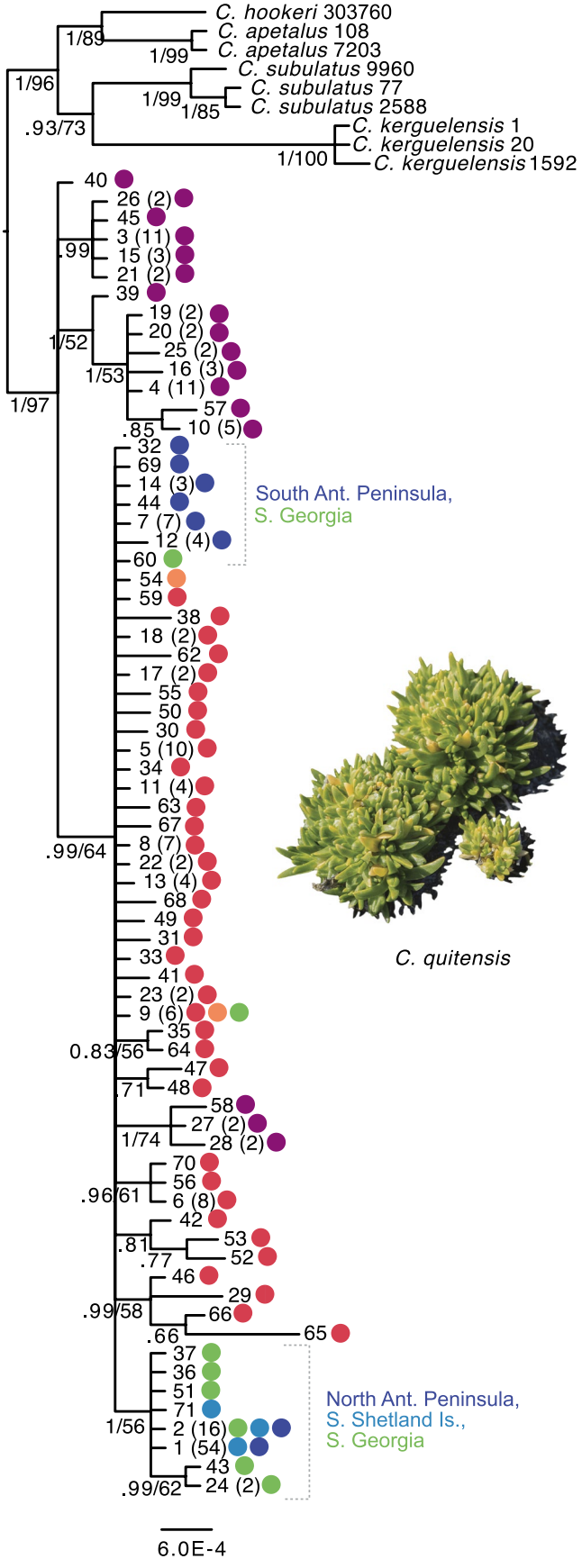

FIGURE 2 (a) Bayesian phylogeny for *Colobanthus quitensis* and outgroup species of unique sequences within the combined *ITS* and *ndhF-rpl32R+rpl32F-trnL* dataset (for list of unique sequences see Table S1). Posterior probabilities and maximum likelihood bootstrap values from MrBayes and RAxML-GUI analyses, respectively, are shown below each node. Colours indicate biogeographical regions shown in Figure 1. Note a disparity between posterior probabilities and maximum likelihood bootstrap support values, likely caused by short branch lengths and/or polytomies (Lewis, Holder, & Holsinger, 2005)

4 | DISCUSSION

4.1 | The origin of *Colobanthus quitensis* in Maritime Antarctica and in South Georgia

Most genetic markers revealed multiple alleles within the Maritime Antarctic (Antarctic Peninsula, South Shetland Islands and South Orkney Islands) that were shared with regions further north (all markers except *atpB-rbcL*; Figure 3), suggesting *C. quitensis* dispersed to the region at least twice, once to the northern Antarctic Peninsula and South Shetland Islands, and once to the southern Antarctic Peninsula (Figure 3d). Both Maritime Antarctic regions shared identical haplotypes with populations from South Georgia, suggesting that these regions, physically separated by ~850–1,300 km, are more closely connected than has been previously thought. The direction in which dispersal events have taken place is not clear. As there is a South Georgian sample in the centre of the TCS network (Figure 3c), it is possible that one or both Maritime Antarctic groups dispersed from South Georgia. However, it is also plausible that the species dispersed from the Maritime Antarctic to South Georgia, especially given the general direction of oceanic and atmospheric currents that characterize this region (Biersma, Jackson, Bracegirdle, et al., 2018). A shared haplotype between populations in southern South America (Patagonia and Falkland Islands) and South Georgia (Figure 3c) suggests that a recent dispersal event from southern South America to South Georgia has also occurred.

Both Maritime Antarctic and South Georgia groups were most closely related (being only one mutational step separated in the compiled cpDNA and nrDNA TCS network; Figure 3c) to the main haplotype comprising Patagonia, the Falkland Islands and South Georgia, and could therefore have originated from any of these latter regions. As both Maritime Antarctic populations share identical haplotypes with South Georgia, and as there are suggestions that the latter may have harboured LGM ice-free refugia (Allegrucci et al., 2006; McCracken, Wilson, Peters, Winker, & Martin, 2013; Van der Putten, Verbruggen, Ochyra, Verleyen, & Frenot, 2010), this location could have been a potential source and refugium for one or both of the Maritime Antarctic populations. This possibility is, however, counter to the general direction of oceanic and atmospheric currents noted above. Alternatively, southern South America could also have been the original source location, with this region being thought to have harboured various Pleistocene

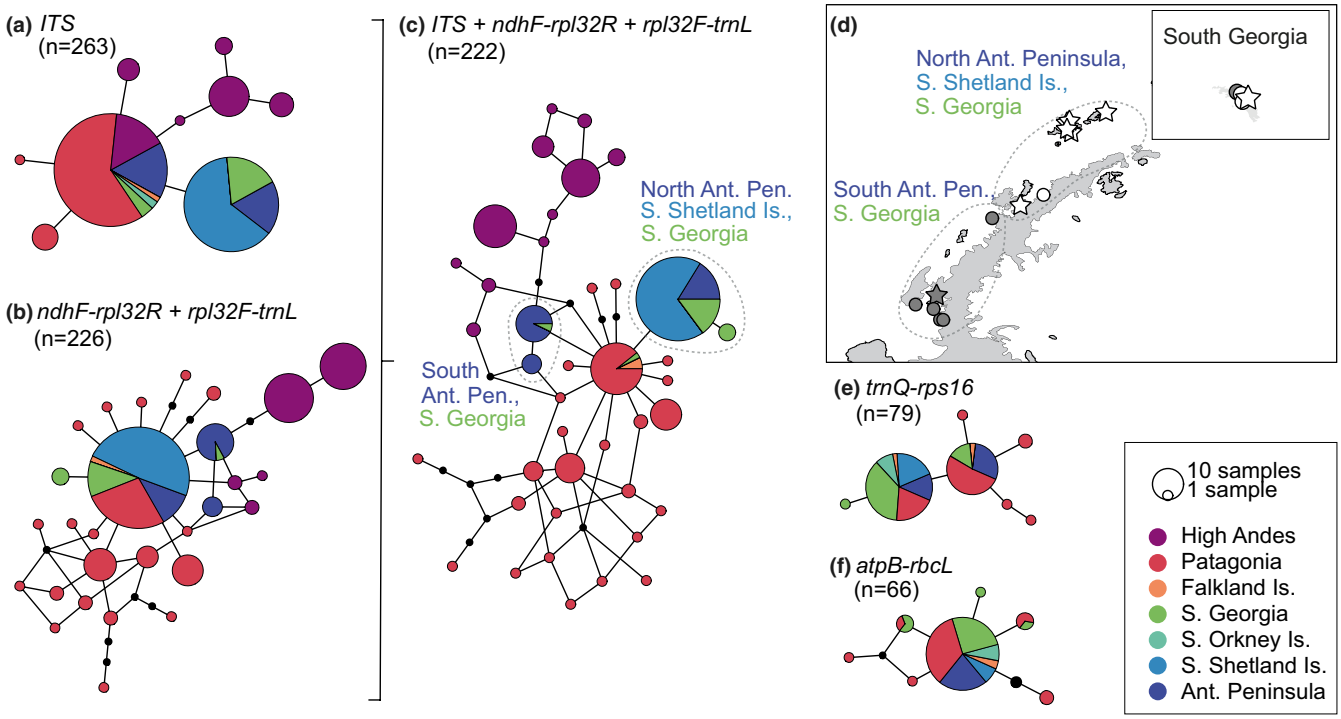

FIGURE 3 TCS genotype and haplotype networks of *Colobanthus quitensis* based on (a) *ITS* and (b) *ndhF-rpl32R+rpl32F-trnL* markers (top and bottom left, respectively), and (c) the *ITS* and *ndhF-rpl32R+rpl32F-trnL* regions combined (right). These analyses include both field-collected and herbarium samples. (d) Map showing sample locations of the two Maritime Antarctic haplotype groups identified in (c) (indicated in (c) with dashed ellipses). Additional TCS haplotype networks of *C. quitensis* of (e) *trnQ-rps16* and (f) *atpB-rbcL* markers, including herbarium samples only. Colours of different biogeographic regions are shown in the key. Stars and circles in (d) indicate field sites and herbarium samples, respectively, as in Figure 1

TABLE 2 Pairwise F_{ST} values (below diagonal) and Φ_{ST} values (above diagonal) among geographic regions of *Colobanthus quitensis* based on the concatenated *ndhF-rpl32R+rpl32F-trnL* dataset. All *p* values were <.00001 (***) or <.01 (**). Values in parentheses indicate numbers of samples

Geographical region	High Andes	Patagonia	South Georgia	South Shetland Is.	Antarctic Peninsula
High Andes (52)	–	.647***	.725***	.866***	.657***
Patagonia (75)	.141***	–	.095**	.179***	.238***
South Georgia (16)	.276***	.208***	–	.359**	.316**
S. Shetland Is. (89)	.644***	.541***	.843***	–	.665***
Ant. Peninsula (29)	.252***	.187***	.344***	.763***	–

refugia (Sersic et al., 2011). Intriguingly, the southern Maritime Antarctic group also showed a close affinity to the northern High Andes populations (separated by only two mutational steps; Figure 3b,c), a finding worthy of investigation in future studies.

4.2 | Recent arrival of the Antarctic vascular flora

Exactly when the dispersal events across the Southern Ocean occurred is not certain, but the shared haplotypes and genotypes with specimens from South Georgia as well as the genetic similarity to specimens from South American regions suggest that *C. quitensis* reached the Antarctic on a relatively recent (late-Pleistocene) timescale. As we report here, previous studies have recorded low genetic variation within *C. quitensis* (Acuña-Rodríguez et al., 2014; Androsiuk et al., 2015; Koc et al., 2018; Lee & Postle, 1975), suggesting a recent spread of the species along the Antarctic Peninsula. This stands in contrast with an earlier suggestion that *C. quitensis* is a likely pre-glacial relict present in Antarctica since the Oligocene–Pliocene (Parnikoza et al., 2007). Notably, we also find that *C. quitensis* is itself a relatively young species (<1 Ma; see Table 3), and much younger than the Oligocene-Pliocene. Our overall results suggest that *C. quitensis* likely only became established in the Maritime Antarctic on a late-Pleistocene timescale, and, although we cannot be certain about its exact arrival time, it possibly only arrived there after the initial post-LGM ice retreat in the Antarctic Peninsula and South Shetland Island regions (*c.* 12–14 ka; Anderson, 2002, and references therein). Our

Method	T_{MRCA} *Sagina – Colobanthus*	T_{MRCA} *Colobanthus*	T_{MRCA} *C. quitensis*
I[a]	3.236 (1.341–5.850) Ma	0.408 (0.112–0.896) Ma	0.181 (0.042–0.431) Ma
II[b]	1.876 (0.911–3.307) Ma	1.450 (0.847–2.137) Ma	0.666 (0.401–0.999) Ma

[a]Based on a previously calculated divergence date for the split between *Colobanthus* and *Sagina* (Dillenberger & Kadereit, 2017).

[b]Based on estimated substitution rate for noncoding chloroplast regions (Yamane et al., 2006).

TABLE 3 Mean estimated time to most recent common ancestor (T_{MRCA}) (95% HDP lower–upper) for *Sagina* and *Colobanthus*, the genus *Colobanthus* and species *C. quitensis*, using two alternative methods for date calibration (see footnotes). All analyses were performed on the combined *ndhF-rpl32R*, *rpl32F-trnL* and *ITS* dataset

inference of multiple successful colonizations of a vascular plant species to the Antarctic over a relatively short time-scale (since late-Pleistocene) is of significance with respect to predicting future colonizations of vascular plants and other organisms on the Antarctic Peninsula, in particular with the increase of ice-free land associated with regional warming (Lee et al., 2017) and recent human activity in the area (Convey & Peck, 2019).

This study fills a significant gap in knowledge of the origin of the Antarctic terrestrial flora. The other native angiosperm, the grass *D. antarctica*, has been the subject of more population genetic studies than *C. quitensis*. For example, Van de Wouw, Dijk, and Huiskes (2008), using amplified fragment length polymorphisms (AFLPs) and chloroplast sequences, detected a low genetic diversity in this grass in the Antarctic, suggesting it was unlikely that *D. antarctica* survived the LGM in Antarctica *in situ*. Subsequently, Fasanella et al. (2017), studying patterns of genetic variability of *D. antarctica* within populations from across both sides of the Drake Passage, identified eight chloroplast haplotypes, of which Antarctic populations included four haplotypes (one unique, the remaining overlapping with Patagonian haplotypes). In the more variable nuclear marker (*ITS*), 17 haplotypes were found in total, of which Antarctic populations included only one haplotype, which was also present in Patagonia. Overall, the results suggested a mid- to late-Pleistocene arrival of the grass in Antarctica, corroborating our findings for *C. quitensis*. Future studies with new markers (such as those identified by Ishchenko, Panchuk, Andreev, Kunakh, & Volkov, 2018; Rabokon et al., 2019) would be useful for clarifying the exact origin of *D. antarctica* in Antarctica. With examples of long-term glacial survival now evident in nearly all Antarctic terrestrial groups (Convey et al., 2008; Convey & Stevens, 2007), including bryophytes (Biersma et al., 2017; Biersma, Jackson, Stech, et al., 2018; Ochyra, 2003; Pisa et al., 2014) and lichens (including many endemic species; Green, Sancho, Türk, Seppelt, & Hogg, 2011; Øvstedal & Smith, 2001), the likely late-Pleistocene arrival of the Antarctic vascular flora is therefore a notable exception to this generalization.

4.3 | Genetic variation of *Colobanthus quitensis* within southern South America

Based on the sampling included in this study, an early split could be found between the majority of the populations from the central South American Andes and those from the remaining populations, including Patagonia (Figure 2). We note that we did not have access to material from populations from areas further north in South America and in southern North America (Mexico), where the species is also sporadically found (see Figure 1b). The high genetic variation and abundance of this species in Patagonia (Table 1; Figure 3a–c) suggests that populations have persisted and remained stable in this region for a long period, and may indicate possible presence within multiple refugia during the Pleistocene, as also found for other species (Sersic et al., 2011).

The southernmost High Andes population sampled (La Parva, near Santiago, Chile) had nine specimens that converged with other more northern High Andes populations, but also five specimens that grouped within the polytomy containing all southern populations (Figure 2). In the haplotype network (Figure 3a), these five samples were equally closely related to southern South American haplotypes and the southern Maritime Antarctic group. The observation that this southern High Andes population at La Parva shares haplotypes with other High Andes populations, as well as with more southerly populations, suggests that there is genetic admixture between the High Andes and Patagonian populations in this region.

With its origin in the Andean range and/or cold regions of Patagonia (see Figure 2), *C. quitensis* is likely pre-adapted to cold, high altitude environments, which are characterized by highly variable conditions (e.g. in temperatures and water availability). The genetic similarity of *C. quitensis* across its current biogeographical distribution suggests ecological niche conservation in its ability to withstand harsh and/or variable conditions (as shown by its tolerance to cold, moderately saline and/or dry environments), combined with opportunistic dispersal capabilities to reach and colonize other suitable habitats (e.g. Antarctic and sub-Antarctic environments). Overall, the genetic information shown here may be useful for future studies that apply niche comparative methods to link macroclimatic variables in explaining the past, present and future distribution of *C. quitensis*.

4.4 | Dispersal within the genus *Colobanthus*

Distribution patterns within *Colobanthus* suggest that the genus is efficient at dispersing to other regions, including across oceans. The outgroup species *C. subulatus* showed identical sequences in samples from Patagonia and South Georgia in all markers (Figure S1.2a–d), suggesting that this species has also recently arrived in South Georgia from Patagonia. Similarly, in *C. kerguelensis*, nearly identical sequences in all markers were found across the remote Kerguelen Islands, Crozet Islands and Amsterdam Island in the Indian Ocean.

While the overall distribution of *Colobanthus* appears Gondwanan (including representatives from New Zealand, Australia, South America, many sub-Antarctic islands and Antarctica), the genus' age is clearly much younger than the break-up of Gondwana (see Table 3 and Dillenberger & Kadereit, 2017), supporting the hypothesis that many *Colobanthus* species are efficient trans-oceanic dispersers. This is confirmed by their presence on various geologically young islands, such as sub-Antarctic Prince Edward Island (*c.* 215 ka; Hänel & Chown, 1998). Further phylogeographical studies are required to assess historical dispersal and speciation patterns within the wider genus.

4.5 | Possible modes of dispersal

While bryophytes and other spore-dispersed biota could have been distributed to Antarctica by wind (e.g. see Biersma, Jackson, Bracegirdle, et al., 2018), the weight of the seeds of *C. quitensis* (~50 μg) probably prevents such dispersal. Oceanic dispersal ('rafting') or animal vectors (e.g. migrating birds) are both more likely routes by which the species could have arrived in Antarctica. The species is known to be moderately salt-tolerant, and in Patagonia, South Georgia and the Maritime Antarctic commonly occurs in many coastal environments, including the top of the intertidal zone in Patagonia (Cuba-Díaz, Castel, Acuña, Machuca, & Cid, 2017). Whether its seeds could survive exposure to seawater during rafting is unknown, but recently an example of rafting kelp has revealed that Antarctica is not completely isolated from biological sea-rafting particles from mid-latitude source populations (Avila et al., 2020).

Another possible mode of dispersal for *C. quitensis* could have been via the plumage of common Antarctic birds, such as gulls (Parnikoza et al., 2012, 2018). However, *C. quitensis* seeds are smooth and have no hooks or spines to facilitate their attachment to bird plumage, lessening the likelihood of this type of trans-oceanic dispersal. Alternatively, dispersal *via* the guts of birds, such as the white-rumped sandpiper (*Calidris fuscicollis*) could have facilitated a historical dispersal event. This long-distance migrating shorebird, breeding in the North American Arctic and wintering in southern South America and the Falkland Islands, is also a rare visitor to South Georgia and the South Shetland Islands (Trivelpiece et al., 1987), where sightings have increased over the last 30 years (Korczak-Abshire, Angiel, & Wierzbicki, 2011). The species has also been observed east of the Antarctic Peninsula (James Ross Island), on the western Antarctic Peninsula, and as far south as Rothera Point, Adelaide Island (Pavel & Weidinger, 2013). In its wintering grounds in southern South America, *C. fuscicollis* feeds on wetlands, shores and saltmarshes. Here, its diet consists mainly of invertebrates, but it also feeds on seeds, including Caryophyllaceae and Poaceae species, which can make up its entire stomach contents (Montalti, Arambarri, Soave, Darrieu, & Camperi, 2003). A rare dispersal event of this seed-foraging shorebird could thus have facilitated the transfer of either or both *C. quitensis* and *D. antarctica* to Antarctica. Future studies are needed to investigate the likelihood of this mode of dispersal.

ACKNOWLEDGEMENTS

We thank Osvaldo J. Vidal for access to the HIP herbarium, Simon Pfanzelt, Bart van de Vijver, Tamara Contador and Javier Rendoll for sampling and/or assistance with sampling, and Markus S. Dillenberger for providing information for the molecular dating analysis. This research was made possible by the logistic support of Instituto Antártico Chileno (INACH) and the British Antarctic Survey. This research was supported by NERC-CONICYT grant NE/P003079/1, Carlsberg Foundation grant CF18-0267, INACH grant RT_11-13 and RG_02-13 and project ALIENANT CTM2013-47381-P granted by the Spanish Ministry of Economy and Competitiveness. The authors declare no conflict of interest. Permits were obtained under the United Kingdom Antarctic Act (S9-29/2014), the Government of South Georgia & the South Sandwich Islands (Permit no. 2017/019) and the Spanish Polar Committee (ASPA 140 Deception Island; project ALIENANT, season 2016). The chief editor, editor and anonymous reviewers gave helpful and constructive comments on the manuscript, for which we are grateful.

DATA AVAILABILITY STATEMENT

Sequence data have been submitted to the GenBank database under accession numbers MN640112–MN640391 and MN614479–MN615128 (see Table S1, Appendix S1). Phylogenetic and Popart matrixes are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.qrfj6q5bw).

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BIOSKETCH

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Author contributions: The first and second authors contributed equally to this paper. M.A.M.M., C.T.D., P.C. and E.M.B. conceived the study; C.T.D., E.M.B, P.C., K.K.N., M.A.M.M., I.S.A.R., M.C.D, M.A.L., G.B. and L.R.P. conducted the field sampling, and E.M.B. conducted the herbarium sampling; E.M.B. and C.T.D. carried out the molecular work; E.M.B., with help from C.T.D. and W.P.G.C., conducted the analyses and wrote the manuscript. All authors contributed significantly to the final manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Biersma EM, Torres-Díaz C, Molina-Montenegro MA, et al. Multiple late-Pleistocene colonisation events of the Antarctic pearlwort *Colobanthus quitensis* (Caryophyllaceae) reveal the recent arrival of native Antarctic vascular flora. *J Biogeogr.* 2020;00:1–11. https://doi.org/10.1111/jbi.13843