INTRODUCTION

The gene flow and phylogeographical patterns of Southern Ocean shallow-water marine invertebrates in general, and sponges in particular, are interesting for a number of reasons. From an evolutionary history perspective, the Southern Ocean provides a unique scenario for studying the impact of drastic environmental shifts on the population dynamics of marine species, with repeated shifts and expansions of ice sheets. Among them, sponges are one of the major components, yet population connectivity of none of their many Antarctic species has been studied. To investigate gene flow, local adaptation and resilience to near-future changes caused by global warming, we sequenced 62 individuals of the sponge *Dendrilla antarctica* along the Western Antarctic Peninsula (WAP) and the South Shetlands (spanning ~900 km). We obtained information from 577 double digest restriction site-associated DNA sequencing (ddRAD-seq)-derived single nucleotide polymorphism (SNP), using RADseq techniques for the first time with shallow-water sponges. In contrast to other studies in sponges, our neutral SNPs data set showed high levels of gene flow, with a subtle substructure driven by the circulation system of the studied area. However, the 140 outlier SNPs under positive selection showed signals of population differentiation, separating the central–southern WAP from the Bransfield Strait area, indicating a divergent selection process in the study area despite panmixia. Fourteen of these outliers were annotated, being mostly involved in immune and stress responses. We suggest that the main selective pressure on *D. antarctica* might be the difference in the planktonic communities present in the central–southern WAP compared to the Bransfield Strait area, ultimately depending on sea-ice control of phytoplankton blooms. Our study unveils an unexpectedly long-distance larval dispersal exceptional in Porifera, broadening the use of genome-wide markers within nonmodel Antarctic organisms.

KEYWORDS
adaptation, ddRADseq, mitochondrial genome, RNA-seq, SNPs, South Shetland Islands
Pliocene–Pleistocene glacial cycles being the major factor in shaping the current diversity and distribution of the Antarctic fauna (Thatje, Hillenbrand, & Larter, 2005). The alternation of glacial and interglacial periods might have especially affected shallow-water benthic invertebrates, eliminating most of the available habitat during glacial maxima (Alcock & Strugnell, 2012; Rogers, 2007; Thatje et al., 2005). These dramatic environmental events left characteristic signatures throughout the genome of these shallow-water invertebrates, most of which have only been assessed using traditional mitochondrial (a fragment of the cytochrome c oxidase subunit I gene [COI]) and nuclear (18S and 28S rRNA genes) markers (e.g., González-Wevar, David, & Poulin, 2011; González-Wevar, Saucède, Saucède, Morley, Chown, & Poulin, 2013; Janosik, Mahon, & Halanych, 2011; Krabbe, Leese, Leese, Mayer, Tollrian, & Held, 2010). Although shallow-water sponges form massive reefs dominating an important fraction of the available hard substrate in Antarctica (Dayton, 1989), no study has yet addressed the population genetics and connectivity of any of the 397 described sponges from this continent (Downey, Griffiths, Linse, & Janussen, 2012; Riesgo, Taboada, & Avila, 2015). To our knowledge, the only study incorporating analyses of the genetic diversity of an Antarctic sponge was conducted on the deep-sea species Stylocordyla chupachups using microsatellites (Carella, Agell, & Uriz, 2019), but we did not consider it as a population genetics and connectivity study because the authors only focused on the sponge clonal reproduction at a very small scale (<2 km).

The Antarctic Peninsula is currently one of the most rapidly warming regions of the planet (Vaughan et al., 2003). The mean atmospheric temperature rose near 3°C during the second half of the 20th century (King, 1994; King & Harangozo, 1998; Turner et al., 2005), with profound consequences for ice sheets and glaciers (Cook, Fox, Vaughan, & Ferrigno, 2005). Moreover, the summer temperature of the surface waters adjacent to the Western Antarctic Peninsula (WAP) increased by more than 1°C during the same period (Meredith & King, 2005), threatening shallow-water Antarctic species, which are less resilient to temperature increases than species elsewhere (Peck & Conway, 2000), and whose essential biological functions are extremely sensitive to temperature fluctuations (Peck, Webb, & Bailey, 2004). This aspect is especially concerning for sponges, as all are sessile organisms known to have lecithotrophic larvae (Maldonado, 2006), which would imply limited dispersal abilities and therefore higher vulnerability (Pascual, Rives, Schunter, & Macpherson, 2017). However, although in the Southern Ocean the reproductive life history stages appear to have little influence in structuring genetic patterns (Halanych & Mahon, 2018), sponge larvae from other latitudes are not usually able to disperse over large distances (Pérez-Portela & Riesgo, 2018), with some exceptions (see Maldonado, 2006). These limited dispersal capabilities generally result in highly structured and isolated populations (Pérez-Portela & Riesgo, 2018), with high levels of inbreeding and a consequently reduced resilience (Botsford et al., 2009). Hence, to assess the degree of resilience that Antarctic sponges will have under future predicted habitat shifts (IPCC 2013th Assessment Report, 2013), it is urgent to investigate their connectivity patterns and gene flow.

Population genetics, which delves into the distribution of genetic diversity within and between populations, depends essentially on the presence of genetic variability to work with. The mitochondrial genome (mitogenome, mtDNA) has been widely used for population genetic and phylogenetic analyses in Metazoan (Avise et al., 1987) due to its high substitution rates (Brown, George, & Wilson, 1979) and its maternal inheritance and haploidy (see Ernster & Schatz, 1981). However, in some early-splitting animal lineages, such as the members of the phylum Porifera, mtDNA variation within and between species is extremely low, due to its slow-evolving nature (Huang, Meier, Todd, & Chou, 2008). With some notable exceptions (DeBiase, Richards, & Shivi, 2010; Duran & Rützler, 2006; López-Legentil & Pawlik, 2009; Xavier et al., 2010), intraspecific relationships in sponges have therefore only been recently addressed using microsatellites (e.g., Blanquer & Uriz, 2010; Calderón et al., 2007; Giles, Saenz-Agudelo, Saenz-Agudelo, Hussey, Ravasi, & Berumen, 2015; Riesgo et al., 2016; Taboada et al., 2018). Within the past few years, new promising approaches for population genetics based on reduced representation genomic libraries combined with high-throughput sequencing techniques, such as restriction-associated DNA sequencing (RADseq) and genotyping by sequencing (GBS), have become routinely implemented in marine invertebrates but hardly on early-splitting lineages (reviewed in Pérez-Portela & Riesgo, 2018). These methods are revolutionizing the ecological, evolutionary and conservation genetic fields because of their power to recover hundreds to thousands of neutral single nucleotide polymorphisms (SNPs) for fine-scale population analyses (Andrews, Good, Good, Miller, Luikart, & Hohenlohe, 2016). However, only one study to date has recovered SNPs in sponges, which used ampolion sequencing to obtain 67 SNPs and detect the small-scale genetic structure of Aphrocallistes vastus (Brown, Davis, & Leys, 2017), the main reef-building glass sponge of the British Columbia continental shelf.

To date, the analysis of RADseq-derived SNPs has just reached Antarctic marine invertebrates with only four studies addressing the population genetic structure of the Antarctic krill Euphausia superba (Deagle, Faux, Faux, Kawaguchi, Meyer, & Jarman, 2015), the brittle stars Ophionotus victoriae (Galaska, Sands, Sands, Santos, Mahon, & Halanych, 2017a) and Astrotoma agassizii (Galaska, Sands, Sands, Santos, Mahon, & Halanych, 2017b), and the sea spider Nymphon austral (Collins, Galaska, Halanych, & Mahon, 2018). Although RADseq data can potentially be used for discovering genomic regions under selective pressure (Catchen et al., 2017; McKinney, Larson, Seeb, & Seeb, 2017), none of the above-mentioned studies has used this approach to delve into the footprints that natural selection and local adaptation left in the genome of the three Antarctic species listed above. In contrast, in other latitudes, RADseq has been successfully used to detect loci under selection, providing the grounds to understand processes of adaptive ecological divergence in a range of nonmodel marine organisms (e.g., Araneda, Larrain, Hecht, & Narum, 2016; Combosch, Lemer, Lemer, Ward, Landman, & Giribet, 2017; Ferchaud & Hansen, 2016; Gleason & Burton, 2016).

The dendroceratid Dendrilla antarctica Topsent, 1905 is one of the dominant sponges inhabiting West Antarctic shallow waters (Sará, Balduzzi, Balduzzi, Barbieri, Bavestrello, & Burlando, 1992),
playing a key role by providing shelter and food for many other marine invertebrates (e.g., Moles et al., 2017). Its distribution spans along the Antarctic Peninsula and its associated islands, to the South Orkney Archipelago as the northernmost point of its range (data from World Porifera Database: www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=164875). *D. antarctica* is a brooding sponge, with yolky lecithotrophic larvae that are released during the Antarctic summer (Koutsouveli et al., 2018). In the present study, we aim to assess the genetic diversity, demographic history, and genetic connectivity of *D. antarctica* at a regional scale in the WAP and South Shetland Islands using double digest (dd)RADseq-derived SNPs. We also evaluate the suitability of the full mitochondrial genome in *D. antarctica* to assess genetic diversity and connectivity. Finally, we test for genetic signatures of divergent selection using SNPs identified in an $F_{ST}$ outlier test, and measure the expression levels of the genes identified under selection in three transcriptome samples spanning the whole latitudinal range of our sampling area.

2 | MATERIALS AND METHODS

2.1 | Sample collection, preservation and DNA extraction

For the population genomics study with ddRADseq, we collected ~1 cm$^3$ of tissue from 67 specimens of *Dendrilla antarctica* during the 2015–2016 austral summer in seven locations across the WAP and the South Shetland Islands (Figure 1; Table 1). Sampling was performed by SCUBA diving at 5–25 m depth. Sponge fragments were preserved in 96% ethanol, with the ethanol being replaced three times, and stored at −20°C until further processing. We extracted DNA from all samples using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturer’s protocol, with minor modifications in the cell lysis time (which was conducted with an overnight incubation) and the final DNA elution step (performed twice using 50 µl of elution buffer each time). DNA quantity was assessed with a Qubit dsDNA HS assay (Life Technologies).

For mitogenome reconstruction, we collected a fragment (~1 cm$^3$) of a specimen in 96% ethanol from Deception Island to perform draft-level genomic sequencing, with genomic DNA (gDNA) extracted as described above. Furthermore, we subsampled tissue fragments (~1 cm$^3$) of three individuals for additional mitogenome reconstruction and transcriptomic analysis, from three different sampling stations (O’Higgins Bay, n = 1; Deception Island, n = 1; and Adelaide Island, n = 1), covering the whole latitudinal range of the sponge in our study. We preserved the subsampled tissue fragments in RNAlater (Life Technologies) immediately after collection, stored them for 24 hr at 4°C, replaced the RNAlater once, and then stored samples at −80°C until further processing.

2.2 | Transcriptomic and genomic library preparation and sequencing

For transcriptomics, total RNA was extracted using a standard trizol-based method using TRI Reagent (Life Sciences) following the manufacturer’s instructions. Subsequent mRNA purification was
performed with a Dynabeads mRNA Purification Kit (Invitrogen) also following the manufacturer’s protocol. Three cDNA libraries were constructed with the ScriptSeq version 2 kit (Illumina), using adapters 9, 10 and 11, and sequenced alongside other samples in a single flowcell of an Illumina NextSeq 500, at 150 bp paired-end read length at the sequencing unit of the Natural History Museum, London (NHM).

Our genomic library for mitogenome recovery was prepared using a TrueSeq DNA PCR-free library kit (Illumina) and sequenced on an Illumina MiSeq at 150 bp nominal paired read length at the sequencing unit of the Natural History Museum, London (NHM).

### 2.3 Transcriptome and mitochondrial assembly, and mitogenome screening

A total of 123,782,845 paired reads (Den_ROT_3 = 29,840,417 reads, Den_OH_2 = 39,434,819 reads, and Den_DEC_19 = 54,507,609 reads) were obtained in our transcriptomic run. A total of 9,644,983 paired raw reads were obtained in our gDNA run, 8,484,436 paired reads after trimming and filtering. Both transcriptomic and gDNA reads were cleaned using Trimmomatic 0.33 (Boiger, Lohse, & Usadel, 2014) with the following settings: ILLUMINACLIP:.../Adapters.fa:2:30:10 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:20 MINLEN:30 where the Adapters.fa file was substituted for the appropriate adapters for each library. Sequence quality was assessed before and after trimming using FastQC (Andrews, 2010) to ensure complete removal of adapter and low-quality sequence data. The final cleaned read files for transcriptomic analyses contained 26,523,504 reads for the sample Den_ROT_3, 30,645,339 reads for the sample Den_OH_2, and 49,611,670 reads for the sample Den_DEC_19.

gDNA reads from the sample from Deception Island were assembled using Velvet 1.2.10 (Zerbino & Birney, 2008) at k-mer sizes of 71 and 91, which were the best k-mers after optimization trials. A local blast database was made from these gDNA assemblies using the makeblastdb command (Altschul et al., 1997). The complete mitochondrial protein-coding sequence for the gDNA sample was obtained by blasting (tblastn) the complete mitochondrial genome of Igernella notabilis (NC_010216) to these assemblies and extracting the best-hit contigs. Reciprocal blastx of the translated nucleotide sequences to the nonredundant database confirmed the homology of these assemblies to Porifera, Dendroceratida.

The three individual transcriptomes were assembled into a de novo reference transcriptome using Trinity version 2013.08.14 (Grabherr et al., 2011) with standard settings except for a minimum contig length of 200 bp and in silico read normalization. This de novo reference transcriptome contained 74,762 transcripts with an N50 of 658 and a total of 38.8 Mb with GC content of 44.9%. Similarly, the three samples were assembled separately using the same Trinity pipeline as above. In addition to the complete mitochondrial genome recovered from the gDNA sample, three more mitochondrial genomes were obtained from the transcriptomic reads or the assembled transcriptomes using the pipeline Trimitomics (Plese et al., 2018). Subsequently, all four mitochondrial genomes (one coming from the gDNA sample and three from the transcriptomes) were aligned in Geneious 8.1.8 (Kearse et al., 2012) using the Q-INS-I algorithm of Mafft version 7 (Katoh & Standley, 2013), which is used as the default algorithm for rRNA alignments because it considers secondary structure information, as a form of base-pairing probability (Katoh & Toh, 2008). The software Dnasp 5.10.01 (Librado & Rozas, 2009) was used to calculate the number of segregating sites (S), haplotype number (H), haplotype diversity (Hd) and nucleotide diversity (π).

### 2.4 ddRADseq library preparation and sequencing

Library preparation was conducted following Peterson, Weber, Weber, Kay, Fisher, and Hoekstra (2012) with the following modifications (as in Combsch et al., 2017). gDNA (100–1,000 ng) was digested using the high-fidelity restriction enzymes Sbf1 and EcoR1 (New England Biolabs). Resulting digested fragments were cleaned with an Apollo 324 (IntegenX) using Agencourt AMPure beads (1.5 × volume ratio; Beckham Coulter), and were subsequently quantified with a Qubit dsDNA HS assay (Life Technologies). Resulting fragments were ligated to custom-made P1 and P2 adapters containing sample-specific barcodes and primer annealing sites. Individually

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**TABLE 1** Collection details for all sampling sites, including abbreviations, coordinates, number of individuals used in the analyses, and date of collection

| Station          | Abbreviation | Coordinates          | Number of individuals | Date of collection |
|------------------|--------------|----------------------|-----------------------|--------------------|
| Deception Island | DEC          | 62°59′25″S 60°37′31″W | 9                     | 13/1/2016         |
| Half Moon Island | HM           | 62°35′41″S 59°54′07″W | 9                     | 24/2/2016         |
| King George Island | KG           | 62°11′55″S 58°56′59″W | 16                    | 21/2/2016         |
| O’Higgins Bay    | OH           | 63°18′52″S 57°54′27″W | 5                     | 19/2/2016         |
| Cierva Cove      | CIE          | 64°09′20″S 60°57′12″W | 9                     | 17/2/2016         |
| Paradise Bay     | PAR          | 64°49′24″S 62°51′24″W | 3                     | 15/2/2016         |
| Adelaide Island  | ADE          | 67°34′04″S 68°08′55″W | 11                    | 12/2/2016         |
| **Total**        |              |                      | **62**                |                    |
barcoded samples were pooled into libraries, cleaned by manual pi-
petting using AMPure beads (1.5 × volume ratio), and size-selected
(270–600 bp) using a Pippin Prep (Sage Science). Each library was
then PCR-amplified using Phusion polymerase with 14–20 PCR cy-
cles (98°C for 10 s, 65°C for 30 s and 72°C for 90 s, with an ini-
tial denaturation step at 98°C for 30 s and a final extension step
at 72°C for 5 min). Resulting libraries were cleaned with an Apollo
324 to remove remaining adapters and primers using AMPure beads
(0.8 × volume ratio). Each library was quantified using a qPCR Kapa
library quantification kit (Kapa Biosystems) and quality-checked
on an Agilent Bioanalyzer 2100 (Agilent Technologies). Libraries were
pooled normalizing their concentration, subsequently pooled with
RNA-seq libraries in the same flowcell, and paired-end sequenced
on an Illumina HiSeq 2500 (Illumina) at the Center for
Systems Biology, Harvard University (Cambridge, MA, USA).

2.5 ddRADseq locus assembly and outlier detection

Quality filtering and locus assembly was conducted with the stacks
pipeline, version 1.44 (Catchen, Hohenlohe, Hohenlohe, Bassham,
Amores, & Cresko, 2013). RAD-tags (DNA fragments with the two
appropriate restriction enzyme cut sites that were selected, ampli-
fied and sequenced) were processed using process_radtags, where
raw reads were quality-trimmed to remove low-quality reads, reads
with uncalled bases, and reads without a complete barcode or re-
striction cut site. The process_radtags rescue feature (-r) was used to
recover minimally diverged barcodes and RAD-tags (--barcode_dist
3; --adapter_mm 2). The process_radtags trimming feature (-t) was
used to trim remaining reads to 120 bp, in order to increase con-
fidence in SNP calling. After performing these filtering steps in
process_radtags, we retained a total of 161,847,986 reads from the
initial 220,380,548 raw reads, with an average of 2,247,889 reads
per sample. Preliminary tests were carried out following Jeffries
et al. (2016) to identify optimal stacks parameters. Final parameter
values were as follows: ustacks: M = 2, m = 3, allowing for gaps (--gapped;
--max_gaps 3; --min_aln_len 0.80), using the removal (-r) and
deleveraging (-d) algorithms; cstacks: n = 4, allowing for gaps
(--gapped; --max_gaps 3; --min_aln_len 0.80); cstacks: allowing for
gaps (--gapped). Mean locus coverage among all samples was 47,435,
ranging from 23,359 to 199,138.

The stacks populations module was used to conduct a first filtering
of the data, retaining those SNPs present in at least 20% of the indi-
viduals (r = 0.2). To prevent the analysis of physically linked loci, and
hence meet the assumptions of subsequent analyses, we used the
"--write_single_SNP" option to retain only the first SNP from each
RAD-tag. A subsequent more accurate filtering was performed using
the adegenet R package (Jombart, 2008; Jombart & Ahmed, 2011;
R Core Team, 2014), assessing SNP distributions across individual
samples and sampling stations, and testing different filtering thresh-
olds in order to maximize the number of retained SNPs and minimize
missing data. This approach provides significant help in defining final
thresholds in comparison with the stacks populations module. Thus,
we finally retained loci present in at least 40% of the individuals, and
filtered out individuals with less than 30% of the final loci, resulting
in a data set containing 577 SNPs and 62 individuals.

In order to differentiate neutral SNPs from putative SNPs under
positive selection, the database containing 577 SNPs was analysed
using default parameters in lositan (Antao, Lopes, Lopes, Lopes,
Beja-Pereira, & Luikart, 2008). We used lositan because it imple-
ments the FDIST2 approach of Beaumont and Nichols (1996), which
provides a robust method when populations deviate from the island
model of migration (Tigano, Shultz, Shultz, Edwards, Robertson, &
Friesen, 2017). Also, it incorporates heterozygosity and simulates a
distribution for neutrally distributed markers (De Mita et al., 2013;
Narum & Hess, 2011). We considered that these features were more
appropriate for our model species studied herein, D. antarctica, than
the characteristics of other Fs−outlier methods. Moreover, we also run
bayescan (Foll & Gaggiotti, 2008) with default parameters, which
only detected one locus under selection already detected by lositan.
We discuss lositan results given the reasons stated above.

2.6 Population genetic analyses

The neutral SNP data set (389 SNPs, 62 organisms, vcf file in
Appendix S1) was used for the population genetic analyses. Genetic
diversity and demographic statistics were calculated using the ape
(Parlis, Claude, & Strimmer, 2004), pegas (Paradis, 2010) and ade-
ogenet R packages. Expected (Hₑ) and observed (Hₒ) heterozygosities
per SNP were extracted and subsequently averaged across sam-
ple within sampling stations from the adegenet "genind" objects,
and then the hw.test function in pegas was used to test for devia-
tions from Hardy–Weinberg equilibrium per SNP. To test whether
D. antarctica populations were in expansion we used the taijima.test
function in pegas to obtain the Tajima’s D statistic for each sampling
site and for the whole set of samples together. To assess inbreeding
within sampling stations and differentiation among them, we used
F₁ₛ and FₛΤ F-statistics respectively, both obtained with the fstat
function in the R package hierfstat (Goudet, 2005).

Population structure was assessed using the function snapclust
in the R package adegenet (Beugin, Gayet, Gayet, Pontier, Devillard,
& Jombart, 2018), the discriminant analysis of principal compo-
nents (DAPC) as implemented in the adegenet R package (Jombart,
Devillard, & Balloux, 2010), and structure version 2.3 (Pritchard,
Stephens, & Donnelly, 2000). Structure and snapclust may produce
similar individual membership probability plots, but they have to-
tally different approaches to the genetic clustering problem: while
structure uses a Bayesian approach with Markov chain Monte
Carlo (MCMC) method to estimate allele frequencies in each clus-
ter and population memberships for every individual, snapclust is a
fast likelihood optimization method combining both model−based
and geometric clustering approaches, which uses the Expectation-
Maximization (EM) algorithm to assign genotypes to populations and
detect admixture patterns. Initial group memberships for snapclust
were chosen using the k-means algorithm (pop.ini = "kmeans"), al-
lowing a maximum K (number of clusters) of 10 (max = 10), and a
maximum number of iterations of 100 (max.iter = 100). The analysis successfully converged at the second iteration. The DAPC analysis was performed by grouping samples by sampling stations, and the number of retained principal components analysis (PCA) axes was chosen using the cross-validation xvalDapc function in the adegenet R package. Structure was run twice, using two distinct data sets: (a) all neutral SNPs (389 SNPs, 62 individuals) and (b) just the neutral SNPs in Hardy–Weinberg equilibrium (210 SNPs, 62 individuals). Both analyses were run for 200,000 MCMC iterations using the admixture model, with a burn-in of 100,000 iterations, setting the putative K (number of clusters) from 1 to 10 with 20 replicates for each run. Structure Harvester (Earl & vonHoldt, 2012) and Clumpp version 1.1.2 (Jakobsson & Rosenberg, 2007) were used to determine the most likely number of clusters and to average each individual’s membership coefficient across the K value replicates, respectively.

Pairwise FST values were estimated to measure the differentiation between pairs of sampling stations using the pairwise.fst function in the hierfstat R package. Their significance was tested with 1,000 permutations using the ade4 R package (Dray & Dufour, 2007). The software BARRIER version 2.2 (Manni, Guerard, & Heyer, 2004) was then used to identify and position genetic breaks in the sampling area. This software uses an improved Monmonier’s algorithm to detect genetic barriers from a matrix of genetic distances (pairwise FST table) linked to a matrix of geographical distances. A Mantel test was performed to test the isolation by distance model, examining the correlation between geographical (accounting for coastlines) and genetic distances, using the mantel.randtest function in the ade4 R package.

To test whether the DAPC grouping or the Barrier’s genetic break explained a significant part of the total genetic variation, two hierarchical analyses of molecular variance (AMOVAs) were performed using the poppr.amova function in the poppr R package.

Finally, to identify gene flow patterns in our study area, Nei’s GST method was used to estimate the relative contemporary migration between sampling stations, using the divMigrate function of the diverSity R package (Keenan, McGinnity, McGinnity, Cross, Crozier, & Prodohl, 2013).

2.7 | Annotation, expression values and structure of putative loci under selection

To improve the annotation step, all RAD-loci containing an outlier SNP under positive selection were mapped back to our de novo reference transcriptome using CLC Genomics Workbench 5.1 local BLAST (Altschul et al., 1997) to obtain the contig for each RAD-locus. All contigs with uniquely mapped loci were then subjected to a BLASTX and BLAT search against nr (default parameters, Altschul et al., 1997), and this annotation was retained for the mapped RAD-locus. A functional annotation analysis was then performed using DAVID 6.8 (Huang Sherman, & Lempicki, 2008a, 2008b). Expression values for putative contigs under selection in each of the three RNA-seq data sets were determined by mapping reads from individual samples to the de novo reference transcriptome using the RSEM package (Li & Dewey, 2011: --aln_method bowtie2) in Trinity (Grabherr et al., 2011). The abundance_estimates_to_matrix.pl script was used to determine comparable values of expression (cross-sample normalization: trimmed mean of M-values, TMM), and the obtained TMM values were subsequently normalized to TPM (transcripts per kilobase million).

To detect signals of geographically divergent adaptive selection, we used the same structuring analyses used for the neutral SNP data set. Hence, STRUCTURE, snapclust and DAPC were run for the data set containing the SNPs under positive selection (140 SNPs, 62 individuals, vcf file in Appendix S2) using the same parameters mentioned above for the neutral data set. Moreover, global FST statistic and pairwise FST values were estimated using the hierfstat and ade4 R packages as specified for the neutral data set. The software BARRIER was also used on the loci under positive selection and, subsequently, two AMOVAs were also performed in the poppr R package to test whether the DAPC grouping or the BARRIER’s genetic break explained a significant part of the genetic variation.

3 | RESULTS

3.1 | Mitogenome diversity

The total length of the mitochondrial genome obtained from our gDNA reads from Deception Island was 19,498 bp, of which 10,949 bp comprised protein-encoding sequences. From our de novo transcriptomes, we were able to recover 10,859 bp for the sample from Adelaide Island, 10,682 bp for the sample from Deception Island and 10,893 bp for the sample from O’Higgins Bay. The alignment of the four sequences encompassed 9,433 bp, with four different haplotypes (H) but only three segregating sites (representing 0.032% of the total) (alignments of the 15 mitochondrial genes in Appendix S3). Specifically, we found one mutation in the gene ATP8 (site 141, T → A) in the transcriptomic sample from Deception Island, one nucleotide varied in the sample from Adelaide Island in NAD4 (site 620, A → C), and finally one nucleotide varied in the gDNA sample from Deception Island in the cytochrome c oxidase I (COI) gene (site 902, T → C). Nucleotide diversity (π) was 0.00016 ± 0.00004 and, because each of the four samples analysed accounted for a different haplotype, haplotype diversity (Hd) was 1.

3.2 | Population genetics analyses using neutral SNPs

Population genetics statistics are shown in Table 2. Tajima’s D values were all negative, showing significant p-values (p < 0.05) in all stations separately and for the whole data set (Table 2). Average expected heterozygosity (Hₑ) ranged from 0.054 in Paradise Bay to 0.142 in King George Island, with a value of 0.162 when all samples were analysed together (Table 2). Average observed heterozygosity (Hₒ) ranged from 0.052 in Paradise Bay to 0.079 in Half Moon Island, with a value of 0.067 for the whole data set (Table 2). The number
of loci not found in Hardy–Weinberg equilibrium ranged from 0 in Paradise Bay to 27 in King George Island, and a total of 178 loci when all samples were treated together (Table 2). Inbreeding coefficient \( F_{IS} \) estimated from heterozygosity \( F_{IS} = 1 - (H_E/H_O) \) ranged from 0.037 in Paradise Bay to 0.616 in Adelaide Island, with a value of 0.586 for the entire data set (Table 2). \( F \)-statistics estimated in the hierfstat R package for all the samples together were \( F_{IS} = 0.595 \) and \( F_{ST} = 0.011 \).

The results of the STRUCTURE analysis for all neutral loci (389 SNPs) are shown in Figure 2a. Although the most likely number of clusters was \( K = 4 \)—inferred from delta \( K \) (Evanno, Regnaut, & Goudet, 2005), shown in Appendix S4a—and many of the samples were assigned to one of the four clusters with a high confidence, there is no clear pattern of geographical structure from these results. In fact, STRUCTURE results may be interpreted as an indication of panmixia or high gene flow among sampled stations. Similarly, snapclust results (Figure 2b) revealed a lack of geographical structure, although the most likely number of clusters for this analysis inferred from Akaike’s information criterion (AIC) was \( K = 2 \) (see Appendix S4b). STRUCTURE results for the data set just containing the 210 SNPs in Hardy–Weinberg equilibrium are shown in Appendix S5. Due to the lack of geographical structure in the results from both data sets, we retained information for all 389 SNPs in Figure 2a.

The two-dimensional representation of the DAPC results taking the first and second DAPC axes showed differentiation of some of the sampling sites (Figure 2c), with O’Higgins Bay and Adelaide Island appearing as the two most divergent stations (see Appendix S6a for the first-third DAPC axes representation, showing a similar population structuring). Adelaide Island is the southernmost site in our sampling area, while O’Higgins Bay represents the northernmost sampled area in the Antarctic Peninsula, which is in fact oceanographically isolated by the Peninsula Front (see Figure 1b).

Pairwise \( F_{ST} \) comparisons showed low to moderate \( F_{ST} \) values ranging from 0 to 0.124 (Table 3, above diagonal). Although all \( F_{ST} \) values were nonsignificant, they allowed us to identify genetic barriers in *Dendrilla antarctica*’s genetic landscape using the BARRIER software. The strongest genetic break separated O’Higgins Bay and King George Island stations from the rest of the sampling localities (Figure 2d). The Mantel test indicated no correlation between the geographical distance matrix and the genetic distance matrix \( (p = 0.687) \), refuting the isolation by distance hypothesis and thus indicating that other factors (e.g., oceanographic features) might be explaining the geographical distribution of *D. antarctica*’s genetic diversity.

The full migration \( G_{ST} \) table is shown in Appendix S7. The highest migration values (>0.8) are represented in the migration network (Figure 3), indicating an isolation of the Central Antarctic Peninsula (Cierva Cove and Paradise Bay), and high contemporary migration between Adelaide Island and the South Shetlands, and within the South Shetlands. High contemporary migration was also detected from O’Higgins Bay to King George Island (Figure 3).

The AMOVA results for the neutral SNP data set are shown in Table 4a. Both the DAPC and the BARRIER groupings appeared to be nonsignificant portions of the genetic variance \( (p = 0.468 \) and 0.248, respectively), the two of them representing less than 1% of the total variation (Table 4a).

### 3.3 Putative loci under selection

A total of 188 \( F_{ST} \) outlier SNPs were detected by LOSITAN, 48 of them identified as under balancing selection and 140 as under positive selection. These 140 SNPs represented 24.3% of the complete SNPs data set.

From the 140 RAD-tags with outlier SNPs under positive selection, 31 matched contigs in our de novo assembled reference transcriptome of *D. antarctica* and for 16 of them we retrieved a Blast hit against the nr NCBI database with e-value \( 1e-05 \) or lower (Table 5). One of them corresponded to an uncharacterized protein, and another one matched a bacterial aminotransferase (Table 5). This low ratio of only one RAD-tag matching a bacterial gene out of the 140 under positive selection (0.7%) is in agreement with previous knowledge on the microbiome of *D. antarctica*, a sponge that is considered to have low microbial abundance (Koutsouveli et al., 2018). For the 14 remaining annotated loci, gene characterization and DAVID
Table 3 Pairwise $F_{ST}$ values for the neutral SNP data set (above diagonal) and the data set composed of putative SNPs under positive selection (below diagonal)

|                  | Cierva Cove | Deception Is. | King George Is. | Half Moon Is. | O’Higgins Bay | Paradise Bay | Adelaide Is. |
|------------------|-------------|---------------|-----------------|--------------|--------------|--------------|--------------|
| Cierva Cove      | 0.102       | 0.077         | 0.114           | 0.119        | 0            | 0.121        |
| Deception Is.    | 0.302       | 0.073         | 0.102           | 0.123        | 0            | 0.106        |
| King George Is.  | 0.210       | 0.141         | 0.084           | 0.073        | 0            | 0.094        |
| Half Moon Is.    | 0.208       | 0.238         | 0.121           | 0.124        | 0            | 0.094        |
| O’Higgins Bay    | 0.421       | 0.280         | 0.150           | 0.167        | 0            | 0.102        |
| Paradise Bay     | 0.204       | 0.063         | 0.099           | 0.167        | 0.017        | 0            |
| Adelaide Is.     | 0.323       | 0.301         | 0.220           | 0.231        | 0.250        | 0.120        |

Note: Significant $F_{ST}$ values ($p < 0.05$) are shown in bold.
The results of the structure analysis for the 140 SNPs under positive selection are shown in Figure 5a. The most likely number of clusters was \( K = 2 \), with \( K = 5 \) as the second most likely number of clusters (inferred from delta \( K \), shown in Appendix S4c). These results indicate a lack of geographical structure in the data set under positive selection, which may be the result of the high migration and gene flow detected in the neutral data set. Similarly, snapclust (Figure 5b) did not retrieve any clear geographical structure for the five clusters inferred from AIC (see Appendix S4d).

The representation of the DAPC results based on the 140 SNPs under positive selection taking the first and second DAPC axes is shown in Figure 5c (see Appendix S6b for the first to third DAPC axes representation). Samples from the Bransfield Strait stations (i.e., South Shetland Islands, O’Higgins Bay and Cierva Cove) were grouped together, while Paradise Bay and Adelaide Island appeared as the most divergent sites (Figure 5c), the latter being the most differentiated sampling station based on the first eigenvalue.

The \( F_{ST} \) statistic for the 140 SNPs under positive selection was estimated at 0.205 in the hierfstat R package. Pairwise \( F_{ST} \) comparisons showed high and mostly significant \( F_{ST} \) values, ranging from 0.017 to 0.421 (Table 3, below diagonal). The most robust genetic break

![FIGURE 3](Image) Contemporary migration network inferred from *divMigrate*. Map of the study area with purple arrows representing the migration values higher than 0.8 in the migration table (see Appendix S7) [Colour figure can be viewed at wileyonlinelibrary.com]

| TABLE 4 | Hierarchical AMOVA results: evaluation of genetic differentiation within and among sampling stations, and within and among the groups inferred from the DAPC and Barrier results for the 389 neutral SNPs data set (a) and the 140 under positive selection SNPs data set (b) |
|---|---|---|---|---|---|
| | df | Sum Sq | Mean Sq | Percentage of variation | p-value |
| (a) Neutral data set | | | | | |
| DAPC: ADE/OH/Rest | | | | | |
| Between DAPC clusters | 2 | 296.97 | 148.48 | −0.15 | 0.468 |
| Between populations | 4 | 610.61 | 152.65 | 0.78 | 0.283 |
| Between samples | 55 | 7,731.82 | 140.58 | 59.21 | 0.001 |
| Within samples | 62 | 2,207.68 | 35.61 | 40.17 | 0.001 |
| BARRIER: KG + OH/Rest | | | | | |
| Between barrier clusters | 1 | 178.45 | 178.45 | 0.61 | 0.248 |
| Between populations | 5 | 729.13 | 145.83 | 0.37 | 0.373 |
| Between samples | 55 | 7,731.82 | 140.58 | 59 | 0.001 |
| Within samples | 62 | 2,207.68 | 35.61 | 40.02 | 0.001 |
| (b) Positive selection data set | | | | | |
| DAPC: ADE/PAR/Rest | | | | | |
| Between DAPC clusters | 2 | 291.76 | 145.88 | 6.72 | 0.043 |
| Between populations | 4 | 370.48 | 92.62 | 4.03 | 0.002 |
| Between samples | 55 | 3,482.35 | 63.32 | 72.38 | 0.001 |
| Within samples | 62 | 409.65 | 6.61 | 16.87 | 0.001 |
| BARRIER: CIE/Rest | | | | | |
| Between barrier clusters | 1 | 128.11 | 128.11 | 1.45 | 0.124 |
| Between populations | 5 | 534.13 | 106.83 | 6.77 | 0.001 |
| Between samples | 55 | 3,482.35 | 63.32 | 74.43 | 0.001 |
| Within samples | 62 | 409.45 | 6.61 | 17.35 | 0.001 |
TABLE 5  Genes (and their corresponding RAD-tags) under positive selection in *Dendrilla antarctica*, alongside BLAST annotations and expression values in the three transcriptome samples

| RAD-tag  | Config in reference transcriptome | Annotation                                                                 | Annotation Abbreviation | E-value       | Expression (TMM-corrected TPM) |
|----------|-----------------------------------|----------------------------------------------------------------------------|--------------------------|---------------|--------------------------------|
| 3332     | TRINITY_DN40585_c0_g1_i1          | Dimethylaniline monoxygenase [N-oxide-forming] 5                           | FMOS5                    | 1.87E-20      | ADE 0 0 1.313                     |
| 7288     | TRINITY_DN35887_c1_g1_i1          | Actin-binding protein IPP-like                                           | IPP                      | 6.05E-42      | OH 12.231 7.113 4.738          |
| 9345     | TRINITY_DN29758_c0_g1_i1          | arf-GAP with GTPase, ANK repeat and PH domain-containing protein 1-like | AGAP1                    | 7.54E-23      | DEC 0.837 0.557 0.948          |
| 11872    | TRINITY_DN30313_c0_g1_i1          | Cilia and flagella associated protein 54                                | CFAP54                   | 5.13E-64      | ADE 0.749 0.807 1.202          |
| 9419     | TRINITY_DN36218_c2_g3_i4          | Death-inducer obliterator 1-like                                         | DIDO1                    | 1.29E-27      | OH 1.927 3.813 4.41           |
| 390      | TRINITY_DN46058_c0_g1_i1          | Dynein heavy chain 3                                                    | DNAH3                    | 6.37E-08      | DEC 0.506 0.865 0.266          |
| 14033    | TRINITY_DN17138_c0_g1_i1          | E3 ubiquitin-protein ligase UBR5                                         | UBR5                     | 4.64E-12      | ADE 0 0 0.242                  |
| 9205     | TRINITY_DN31666_c0_g1_i3          | Glycylpeptide N-tetradecanoyltransferase 1                              | NMT1                     | 5.13E-64      | OH 0 0 0.446                   |
| 1845     | TRINITY_DN36199_c4_g2_i2          | Kelch domain-containing protein 8A                                       | KLDHC8A                  | 1.12E-52      | DEC 2.059 0.411 6.931          |
| 2166     | TRINITY_DN36199_c4_g2_i4          | Kelch domain-containing protein 8A                                       | KLDHC8A                  | 6.46E-45      | ADE 3.743 1.892 9.235          |
| 7585     | TRINITY_DN42392_c0_g1_i1          | Pleiotropic regulator 1-like                                            | PLRG1                    | 2.27E-29      | ADE 1.343 0 0                 |
| 2069     | TRINITY_DN17268_c1_g1_i1          | Putative scavenger receptor cysteine-rich protein type 12 isofrom X1    | CD163L1                  | 2.35E-38      | ADE 0.275 0.411 0.502          |
| 2347     | TRINITY_DN27486_c0_g1_i1          | RING finger protein 157                                                 | RNF157                   | 5.13E-64      | ADE 1.706 1.76 4.602           |
| 3110     | TRINITY_DN27486_c0_g1_i1          | RING finger protein 157                                                 | RNF157                   | 2.01E-63      | ADE 1.706 1.76 4.602           |
| 6870     | TRINITY_DN36204_c5_g4_i2         | rRNA intron-encoded homing endonuclease                                 | PAE1850                  | 1.92E-60      | ADE 12,432.867 11,436.449 4,836.602 |
| 4535     | TRINITY_DN34338_c2_g1_i2          | SH3 domain-containing kinase-binding protein 1-like isofrom X2           | SH3KBP1                  | 2.71E-13      | ADE 0 0 9.65                 |
| 2011     | TRINITY_DN46934_c0_g1_i1          | Uncharacterized protein                                                 | —                        | 1.25E-61      | ADE 0.363 0 0.074              |
| 9873     | TRINITY_DN25724_c0_g1_i1          | Aminotransferase BACTERIA                                               | —                        | 5.13E-64      | ADE — — —                     |

Abbreviations: TMM, trimmed mean of M-values; TPM, transcripts per kilobase million.
determined by the barrier software using these pairwise $F_{ST}$ values separated Cierva Cove from the rest of the stations (Figure 5d).

AMOVA results for the “under positive selection” data set are shown in Table 4b. The barrier grouping isolating Cierva Cove (Figure 5d) represented a nonsignificant 1.45% of the total variation ($p = 0.124$). On the other hand, the DAPC clustering separating Adelaide Island, Paradise Bay and the Bransfield Strait stations (Figure 5c) reached a significant 6.72% of the total genetic variance ($p = 0.043$).

4 | DISCUSSION

4.1 | Mitogenome diversity

Our study unveiled an extremely low mitochondrial diversity in *Dendrilla antarctica*, with only four individual SNPs across 9,433 bp of protein-coding mitochondrial sequence data. Although mitogenome sequences were only obtained from four individuals, the low nucleotide diversity we observed in organisms spanning the whole latitudinal range of our sampling area (~900 km, which is almost the entire species distribution) indicates that protein-coding mitochondrial markers provide almost no resolution for population genetic studies for *D. antarctica*. This extremely low variability in mitochondrial markers is not uncommon in sponges because COI has traditionally showed relatively low genetic variation at both intra- and interspecific levels (e.g., Dailianis, Tsigenopoulos, Dounas, & Voultsiadou, 2011; León-Pech, Cruz-Barraza, Cruz-Barraza, Carballo, Calderon-Aguilera, & Rocha-Olivares, 2015; Riesgo et al., 2016; Sentiawan et al., 2016; Taboada et al., 2018) with just a few exceptions (DeBiasse et al., 2010; Duran & Rützler, 2006; López-Legentil & Pawlik, 2009; Xavier et al., 2010), probably due to slower rates of mitochondrial genome evolution and/or the presence of active mitochondrial repair mechanisms (Huang, Meier, et al., 2008).

4.2 | Population genomic analyses using neutral SNPs

In contrast with the low variability of the mitochondrial genome in *D. antarctica*, our study revealed a high resolution power of ddRAD-seq-derived SNPs for population genetic studies. The analyses of our 389 neutral SNPs showed the characteristic signatures of a complex evolutionary history, probably the result of consecutive demographic shifts due to glacial cycles. For instance, significantly negative Tajima’s $D$ values were found in all sampling stations and in the whole data set (Table 2), indicating a deviation in the haplotype frequencies from the neutrality model (Tajima, 1989). These results support the existence of a recent and rapid demographic expansion of *D. antarctica* in the WAP and the South Shetlands, which could have started after the last glacial–interglacial alternation (~20,000–10,000 years ago) when the last Antarctic shelf recolonization took place (see Alcock & Strugnell, 2012). This hypothesis has been suggested for other shallow-water Antarctic invertebrates (Díaz, Féral, Féral, David, Saucède, & Poulin, 2011; González-Wevar et al., 2011; Leiva, Riesgo, Riesgo, Avila, Rouse, & Taboada, 2018; Thornhill, Mahon, Norenburg, & Halanych, 2008), which could have migrated northwards to sub-Antarctic islands during glacial periods and recolonized the Antarctic shelf during interglacial periods. This expansion–contraction model has already been tested for the Antarctic limpet *Nacella concinna* (Strebel, 1908), demonstrating its glacial survival in the sub-Antarctic South Georgia Island, followed
by post-glacial recolonization of the Antarctic Peninsula shelf (González-Wevar et al., 2013).

Taking expected heterozygosity ($H_E$) as a measure of genetic diversity, as originally defined (Nei, 1973), we found significantly lower genetic diversity values for *D. antarctica* (Table 2: $H_E = 0.162$ ranging from 0.054 to 0.142) than those reported for similar population genetic studies on sponges using microsatellite markers (average $H_E$ ranging from 0.4 to 0.8; see Pérez-Portela & Riesgo, 2018), and lower than the $H_E$ values (from 0.24 to 0.323) reported in the only previously published study using SNPs in sponges (Brown et al., 2017). In other examples using SNPs in different animal phyla, $H_E$ ranged from 0.298 to 0.312 in the salmon louse *Lepeophtheirus salmonis* (Jacobs et al., 2018), from 0.211 to 0.214 in the Galapagos shark *Carcharhinus galapagensis* (Pazmiño, Maes, Maes, Simpfendorfer, Salinas-de-León, & Herwerden, 2017), or from 0.128 to 0.276 in the sea anemone *Nematostella vectensis* (Reitzel, Herrera, Herrera, Layden, Martindale, & Shank, 2013), thus corroborating our low values for *D. antarctica*. The extremely low genetic diversity of *D. antarctica* could be related to the particular evolutionary history of the shallow-water Antarctic benthic fauna, a consequence of the bottleneck events affecting benthic species during glacial periods. These demographic events dramatically reduce genetic diversity after

**FIGURE 5** Genetic structure results based on the 140 outlier SNPs under positive selection. (a) **STRUCTURE** results with $K = 2$ and $K = 5$, the first and second most probable number of clusters, respectively (see delta $K$ plot in Appendix S4), (b) **snapclust** results with $K = 5$ (see AIC plot in Appendix S4), and (c) **DAPC** results: two-dimensional representation of the first (horizontal axis) and second (vertical axis) PCA eigenvalues. (d) Map of the study area with the genetic break from the **barrier** analysis shown as a red dashed line. See abbreviations in Figure 1 [Colour figure can be viewed at wileyonlinelibrary.com]
population decimations, as has already been reported for other shallow-water Antarctic fauna (see Allcock & Strugnell, 2012).

Our results revealed high admixture and lack of population differentiation, supported by the low global $F_{ST}$ of 0.011 and the nonsignificant pairwise $F_{ST}$ values (Table 3), suggesting high connectivity and dispersal capability of *D. antarctica* throughout the sampling area, which covered most of the species distribution. We propose that this could be due to the relatively long planktonic life of *D. antarctica* larvae as a result of the great amount of proteinaceous yolk that they contain (Koutsovou et al., 2018) in comparison with sponge larvae from congeneric species from lower latitudes (e.g., Ereskovsky & Tokina, 2004). Furthermore, the strong oceanic currents in the study area (Moffat, Beardsley, Owens, & Van Lipzig, 2008; Zhou, Niier, & Hu, 2002; see Figure 1b) may increase the dispersal ability of *D. antarctica* larvae. Remarkably, our results differ from most previous population genetic studies on sponges, which generally report highly structured and differentiated populations, even at local and regional scales (e.g., Brown et al., 2017; DeBiasse et al., 2010; Pérez-Portela, Noyer, & Becerro, 2015; Riesgo et al., 2016). Even compared to some oviparous sponges such as *Cliona delitrix* which appears to disperse along the ~315 km of the Florida reef tract (Chaves-Fonnegra, Feldheim, Secord, & Lopez, 2015), our results suggested an unprecedented ~900 km contemporary migration. However, although this long-distance connectivity is unusual in sponges, it is common in other Antarctic marine invertebrates. Examples of high gene flow are shown in many Antarctic species, such as the brittle star *A. agassizii* (Galaska et al., 2017a) and *Ophionotus victoriae* (Galaska et al., 2017b), the Antarctic limpet *N. concinna* (González-Wevar et al., 2013), the nemerteans *Parborlasia corrugatus* (Thornhill et al., 2008) and the annelid *Pterocirrus giribeti* (Leiva et al., 2018).

In agreement with STRUCTURE and snapclust results, relatively high gene flow was detected in our contemporary migration network between the South Shetlands and Adelaide Island (Figure 3). We propose that these high migration values are a consequence of the Antarctic Peninsula Coastal Current (APCC) running southwards off the WAP (Moffat et al., 2008; Figure 1b) and the ACC running northwards, connecting stations ~900 km apart. Other high migration values were found within the South Shetlands Archipelago, and from O’Higgins Bay to King George Island (Figure 3). This result could be explained by different factors that may be occasionally weakening the Peninsula Front, an oceanic front produced by the intrusion of a tongue of water from the Weddell Sea in the Bransfield Strait (Sangrà et al., 2011; Figure 1b). For instance, its seasonality is not completely understood yet, due to the sampling season solely extending during austral summer (Hunek, Huhn, & Schröder, 2016; Sangrà et al., 2011; Zhou, Niier, Zhu, & Dorland, 2006). Also, the interfrontal anticyclonic eddy system found between the Peninsula Front and the Bransfield Current (Sangrà et al., 2011) could be potentially interfering with the impermeability of the Peninsula Front. Moreover, the Southern Annular Mode (SAM) and El Niño Southern Oscillation (ENSO) have been found to play a role in the water masses distribution of the Bransfield Strait (Dotto, Kerr, Mata, & Garcia, 2016; Barlett, Tosonotto, Tosonotto, Piola, Sierra, & Mata, 2018, respectively), which may cause interannual variation of the Peninsula Front. Interestingly, our contemporary migration network also showed that the most disconnected region in our study area was the centre of the WAP, where both Cierva Cove and Paradise Bay sampling sites are located (Figure 3). The oceanic features of our study area could also be behind the isolation of this region, which is disconnected from other areas by the Peninsula Front in the north and by the APCC in the west, running through the western side of the Palmer Archipelago (Moffat et al., 2008; Figure 1b).

Despite the lack of strong population structure, DAPC detected slight patterns of population differentiation between O’Higgins Bay, Adelaide Island and the remaining sampling stations. This differentiation could be driven by the contemporary oceanographic features in the study area, with Adelaide Island as the southernmost sampling site and O’Higgins Bay representing the area at the tip of the WAP isolated by the Peninsula Front. Accordingly, the main genetic break we detected partially coincided with the Peninsula Front, but grouping King George Island together with O’Higgins Bay (Figure 2d), which is in agreement with the high migration flow from O’Higgins Bay to King George Island discussed above. A similar genetic break coincident with the Peninsula Front has already been identified for the brittle star *O. victoriae* using SNPs (Galaska et al., 2017a), and also for the intertidal phyllophocid *P. giribeti* using a fragment of the mitochondrial COI marker (Leiva et al., 2018). In addition, different reproductive timing due to, for instance, the effects of north–south differences in sea-ice retreat (Stammerjohn, Martinson, Smith, & Iannuzzi, 2008) could also play a role in population substructure with distinct breeding groups (Sugg, Chesser, Dobson, & Hoogland, 1996).

However, both DAPC and barrier groupings appeared as a nonsignificant part of the total genetic variation in the AMOVAs (Table 4a). This may be due to the high admixture and migration detected in the neutral data set (Figures 2a,b and 3), and hence we suggest that both groupings should be understood as permeable barriers.

### 4.3 Signals of divergent adaptive selection

In our SNP data set we identified 140 outlier SNPs as candidates for positive selection. Based on this data set, we recovered a high $F_{ST}$ statistic value of 0.205, along with high and significant pairwise $F_{ST}$ values (Table 3, below diagonal), revealing geographically divergent adaptive selection. In species with high levels of population connectivity, such as *D. antarctica* here, local adaptation requires high levels of divergent selection. This has already been reported for other marine invertebrates with planktonic larvae, such as the marine snail *Chlorostoma funebralis* (Gleason & Burton, 2016) and the red abalone *Haliotis rufescens* (De Wit & Palumbi, 2013), both from the Pacific coast of California in the USA. Other examples from fish with a similar pattern are the Atlantic cod *Gadus morhua* (Barth et al., 2017) and the Atlantic herring *Clupea harengus* (Limborg et al., 2012). These results are particularly relevant for the Southern Ocean, as they challenge the classic consideration of Antarctic organisms as stable and homogeneous along their distributions.
The structure and snapclust results from the 140 SNPs under positive selection also showed the effects of the admixture and high migration discussed above in the neutral data set (Figure 5a,b). However, we observed two unique genetic clusters at the central and southern WAP (Paradise Bay and Adelaide Island), one of them appearing at both stations (purple individuals in Figure 5b) and the other one exclusively present at Adelaide Island (blue individuals in Figure 5b). In agreement, the DAPC analysis clustered together all the stations from the Bransfield Strait area, separating Adelaide Island (as the most divergent sampling station) and Paradise Bay (Figure 5c). The significant 6.72% of the variance explained by this DAPC grouping (Table 4b) suggests different selective pressures in the central–southern WAP (Adelaide Island and Paradise Bay) and in the Bransfield Strait area (remaining sampling stations).

In this scenario with divergent selective pressures promoting local adaptation, we identified the function of the genes with signatures of selection, with some of them related to the organization of the cytoskeleton (Figure 4). Two of these genes, dynein heavy chain 3 (DNAH3) and cilia and flagella associated protein 54 (CFAP54), are involved in the assembly, function, motility and power stroke of flagella and cilia (Asai & Koonce, 2001; Carter, 2013; McKenzie et al., 2015). As with most other sponges, D. antarctica is a filter-feeding sponge that relies on flagellar beating to modulate the inflow current for particle feeding, and therefore we suggest that the selection signatures in the previously mentioned genes might be related to divergent filtering abilities between the Bransfield Strait area and the central and southern WAP. Furthermore, in general terms, cytoskeletal elements are involved in the regulation of many cellular functions related to immune response, such as cell migration, antigen recognition and phagocytosis (Vicente-Manzanares & Sánchez-Madrid, 2004). The gene actin-binding protein IPP-like (IPP) plays a role in organizing the actin cytoskeleton (Ciobanasu, Faivre, & Le Clainche, 2013), which is essential for immune responses (Wickramarachchi, Theofilopoulos, & Kono, 2010). In addition, heavy chain dyneins such as DNAH3 have also been reported to aid in the formation of stress granules (SGs) (Kwon, Zhang, & Matthias, 2007; Loschi, Leishman, Berardone, & Boccaccio, 2009). SGs are cytosolic aggregations consisting of RNAs and RNA-binding proteins which appear in response to different stressors, with important function in preserving mRNA and regulating its translation during stress responses (Kedersha & Anderson, 2002). In addition, SGs also prevent apoptosis (e.g., Buchan & Parker, 2009), contain antioxidant machinery (Takahashi et al., 2013), and are involved in cellular recovery after stress exposure (Kedersha et al., 2002). Gleason and Burton (2016) found a heavy chain dynein under positive selection in the marine snail C. funebris, relating the selective pressure in this locus to the formation of SGs and their function during thermal stress.

Furthermore, four other genes with functions related to apoptosis appeared under positive selection throughout our sampling area (Figure 4). Apoptosis is a conserved mechanism that occurs during antibacterial responses in sponges (Wiens et al., 2006). Da Fonseca, Kosiol, Kosiol, Vinař, Siepel, and Nielsen (2010) reviewed previous studies on selective pressure in apoptosis-related genes, concluding that positive selection in apoptotic genes is caused by their immune function. Indeed, some of the other genes under positive selection in our data set were related to ubiquitination (Figure 4), a function also related to the immune system, as it regulates the pattern-recognition receptor signalling that mediates immune responses (Hu & Sun, 2016). Moreover, ubiquitination has been related to local adaptation in corals, where it responds to different environmental factors that cause stress (Bay & Palumbi, 2014; Jin et al., 2016; van Oppen et al., 2018). This is due to its role in removing macromolecular debris such as reactive oxygen species (ROS) generated by cellular stress (Kültz, 2003). Interestingly, dimethylaline monooxigenase 5 (FMO5), the enzyme resulting from one of the genes under positive selection retrieved here for D. antarctica, catalyses the oxygenation of N,N-dimethylanilines, a reaction present in the ROS biological detoxification pathway (Jakoby, Bend, & Caldwell, 2012). Furthermore, CD163, which also appeared to be under positive selection here, is associated with the immune system and the response to environmental stressors as well (Figure 4; Burkard et al., 2017; Fabriek et al., 2009). Finally, two RNA post-transcriptional modification genes were identified as under positive selection (Figure 4). This mechanism aids gene regulation under various cellular stress situations (e.g., Anderson & Kedersha, 2009; Chinnusamy, Zhu, & Zhu, 2007; Filipowicz, Bhattacharyya, & Sonenberg, 2008; Floris, Mahgoub, Mahgoub, Lanet, Robaglia, & Menand, 2009).

The signatures of selection in stress and immune responses that we detected are mostly related to the molecular toolkit that sponges, which are generally filter-feeders, use to discriminate between, and react to, food, pathogens and symbionts in the seawater that they filter and that runs through their bodies (Pita, Hoeppner, Ribes, & Hentschel, 2018). Hence, different microbiome components in the seawater in different areas would elicit divergent adaptive strategies in sponges in the particular genes that we detected here as under positive selection. Interestingly, differences in sea-ice duration in the Antarctic Peninsula’s shallow waters usually translate into highly divergent seawater microbiota, both in composition and in abundance (Ducklow et al., 2013; Vernet et al., 2008). While total sea-ice duration in the vicinity of Adelaide Island is around 250 days a year, with a summer sea-ice retreat, total sea-ice duration is below 150 days in the other sampling stations, generally with spring retreats (Stammerjohn et al., 2008). This difference in sea-ice duration is key to maintaining vastly different planktonic communities between the southern WAP and the Bransfield Strait area, because the presence and magnitude of phytoplankton blooms in the Southern Ocean are regulated by the timing of sea-ice retreat (Ducklow et al., 2013; Luria, Ducklow, & Amaral-Zettler, 2014; Vernet et al., 2008). Generally, the later the sea-ice retreats, the higher the phytoplankton productivity, as a consequence of sea-ice inhibition of the formation of a spring deep mixed layer, which in turn inhibits phytoplankton (Ducklow et al., 2013). Furthermore, phytoplankton–bacteria trophic coupling has been demonstrated in the Antarctic Peninsula by the direct bacterial assimilation of recent photosynthetic products (Morán & Estrada, 2002; Morán, Gasol, Pedrós-Alió, ...
& Estrada, 2001) and by the bacterial dependence on dissolved organic matter, which in turn depends on phytoplankton (Ducklow et al., 2013). Apart from the effects on the planktonic communities, a later sea-ice retreat produces fresher and colder summer surface waters in the southern WAP, due to more recent or ongoing seasonal ice melting (Ducklow et al., 2013).

Thus, due to the ice–plankton interaction outlined above, phytoplankton and bacterial communities, as well as summer surface water temperature, differ widely between the southern WAP, and the Bransfield Strait area. Because sponges are able to feed on both diatoms and bacteria, the different composition of these communities across our study area could potentially drive local adaptation of *D. antarctica* populations, not only because of their relevant role in food availability, but also as potential agents of diseases and other stresses. Further studies will be directed to test whether this local adaptation hypothesis we suggest for *D. antarctica* is a general pattern also present in other benthic filter-feeding invertebrates sampled in the same studied area, or even whether microplankton composition generally drives the adaptation of sponges.

A comparison of normalized expression values for the 14 annotated genes under positive selection showed that most of the variation occurred between the sample from Deception Island and the other two samples (Table 5; Figure 4). This contrasts with the DAPC results of the neutral and under positive selection data sets (Figures 2c and 5c), and it is probably because of the physicochemical particularities of the waters of Deception Island, which is an active volcano with a submerged caldera (Port Foster) where our samples were collected. The waters of Port Foster are characterized by the presence of suspended volcaniclastic particles (Baldwin & Smith, 2003) and chemicals from local geothermal activity (Deheyn, Gendreau, Baldwin, & Latz, 2005; Elderfield, 1972). Moreover, the fumarolic emissions and geothermal springs spotting the sedimentary seafloor confer upon Port Foster unusually high bottom-water temperatures of 2–3°C (Ortiz et al., 1992). These features undoubtedly affect and stress benthic filter feeders such as *D. antarctica*, and may have contributed to the upregulation of genes related to different stresses. Proper differential gene expression analyses will be conducted to test whether particular physicochemical water features at Deception Island are determinant in shaping gene expression in a wide array of shallow-water invertebrates, thus testing their adaptation potential at the transcriptome level.

## 5 | CONCLUSIONS

Overall, the current gene flow scenario for *Dendrilla antarctica* is characterized by high migration and low population differentiation, with a subtle population substructure driven by the oceanic features of the region. Remarkably, despite this background of population admixture, we identified divergent selective pressures along the studied region that could be explained by the sea-ice–benthos coupling via planktonic communities. Local adaptation was long assumed to be erased when high population connectivity was present in marine organisms. However, recent investigations indicate that even though few larvae might suffice to maintain genetic homogeneity between populations, that is hardly possible for loci under selection (Sanford & Kelly, 2011). The implications of our results are therefore vast. Our relatively slight patterns of local adaptation are indicative of the potential for plastic physiological responses to environmental shifts. In addition, and in contrast to previous studies of shallow-water sponges, we report a well-connected network of populations across ~1,000 km. Our study therefore corroborates that populations that appear homogeneous for neutral loci may also exhibit local adaptation. In this sense, our study suggests a finely tuned physiological response to current conditions but high resilience to future changes for *D. antarctica* in the Antarctic Peninsula. However, due to larval reliance on oceanic currents to maintain high dispersal abilities, this exceptional gene flow might be threatened by changes that increasing sea temperature could create in Southern Ocean oceanographic circulation patterns, which are not completely understood yet (Meijers, 2014). Moreover, a general reduction of planktonic larval duration is expected for all larvae in the near future, because their metabolic, developmental and growth rates are determined by water temperature (O’Connor et al., 2007). Thus, a shorter larval stage would imply a reduction of the dispersal capabilities of *D. antarctica*, with implications for its gene flow and resilience, due to a putatively higher proportion of larvae dying before reaching a suitable settlement site, as has been proposed for fish larvae (Kendall, Poti, Poti, Wynne, Kinlan, & Bauer, 2013; O’Connor et al., 2007). Therefore, our results can be used as a baseline for future assessments of the effects of a changing Southern Ocean on the population connectivity and resilience of *D. antarctica*.

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## AUTHOR CONTRIBUTIONS

C.L., S.T., G.G. and A.R. conceived and designed the study; C.L. and G.G. conducted fieldwork and collected samples; C.L., S.T., D.C. and
N.J.K. conducted laboratory work; C.L., S.T., T.J., N.J.K. and A.R. performed statistical analyses and interpreted the results; C.L., S.T. and A.R. wrote the manuscript, and all authors edited various versions of the manuscript.

DATA ACCESSIBILITY

RAD-seq data for each individual sample are deposited in the NCBI SRA database, BioProject PRJN531366, Biosamples SAMN11350306–SAMN11350367 (Leiva et al., 2019). Data of the three transcriptomes are deposited in the same BioProject under accession numbers SRR8886798, SRR8886808 and SRR8886813. Alignments of the 15 mitochondrial genes and vcf files of both the neutral data set and the SNPs under positive selection are found in Appendices S1–S3.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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