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ORIGINAL PAPER

Single nucleotide polymorphism data reveals distinct geographic structuring in the Antarctic circumpolar sea spider *Nymphon australe*

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Abstract

The Antarctic benthos is rich in biodiversity, with many species being endemic to the Southern Ocean. Multiple factors such as oceanic currents, glacial cycles and reproductive life stages have been attributed to the distribution of benthic dwelling invertebrates around the continent. The sea spider (Pycnogonida) *Nymphon australe* is a paternal brooder, which lacks a pelagic planktonic life stage. Typically brooding is assumed to suggest limited dispersal capabilities. Here we investigated the genetic structure of *N. australe*, a highly abundant pycnogonid species in the Southern Ocean to test assumptions of a documented circumpolar distribution. Previous studies with mitochondrial data have revealed that *N. australe* has high genetic diversity, limited gene flow, as well as distinct geographic structure. To resolve the phylogeographic structure of the circumpolar *N. australe* from the Antarctic continental shelf, we used 3RAD single nucleotide polymorphism (SNP) data from 111 individuals sampled from ten different, circumpolar geographic regions including the Western Antarctic Peninsula, Ross Sea, Weddell Sea, and Eastern Antarctica. Analyses revealed populations to have distinct regional populations with strong geographic structuring observed by locality and suggest the possibility that *N. australe* may be a species complex in the Southern Ocean.

Keywords Pycnogonid · 3RAD · Population genomics · Antarctica · Phylogeography · RAD-seq

Introduction

The Southern Ocean is home to many unique and highly endemic benthic communities (Aronson et al. 2007; Clarke 2008; Dömel et al. 2020). This high level of endemism has been linked to the ocean's geological history including isolation, cooling and glacial-interglacial cycles (Baird et al. 2021). In the past, the Antarctic Circumpolar Current (ACC) has been indicated as a vector for distribution of larval and or adult life stages of many organisms, and thus been the explanation for the dispersal of many species with circumpolar distribution around the continent of Antarctica (Gibson and Atkinson 2003; Wilson et al. 2007; Linse et al. 2007; Hoffman et al. 2012; Galaska et al. 2017b, a; Halanych and Mahon 2018; Leiva et al. 2018, 2019; González-Wevar et al. 2019; Levicoy et al. 2021). However, recent research has shown that Antarctic currents closer to shore such as the Weddell Sea Gyre, Ross Sea Gyre, Antarctic Slope Current (ASC), Antarctic Peninsula Coastal Current (APCC) and Circumpolar Deep Water (CDW) add complexity in predicting geographic dispersal capabilities (Galaska et al. 2017a; Halanych and Mahon 2018). Additionally, glacial cycles have been noted to play a role in shaping the Southern Ocean's biodiversity and community structure (Thatje et al. 2005). Glacial maxima forced benthic communities on the continental shelf into deeper waters or pockets of refuge, while times of glacial contraction exposes new habitat, ultimately shaping the benthos of the Southern Ocean (Thatje et al. 2005; Clarke 2008). These forces and systems have complex interactions with one another, allowing for a multitude of explanations regarding the dispersal and population structure for species residing in the Antarctic.

Past population genomic analyses have observed phylogeographic structuring in Antarctic benthic invertebrates such as echinoderms, crinoids, molluscs, bivalves, annelids,

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and sponges. Mitochondrial data revealed the brittle star *Astrotoma agassizii* to be genetically discontinuous across the polar front, with populations previously assumed to be panmictic representing three separate lineages, all lacking morphological distinction (Hunter and Halanych 2008). A follow-up study on this species utilizing single-nucleotide polymorphism data confirmed the existence of strongly divergent populations on either side of the Drake Passage and provided evidence of recent genetic contact between South America and the Southern Ocean (Galaska et al. 2017a). A study examining genetic structure of the brittle star *Ophionotus victoriae* utilizing SNP data revealed geographically distinct populations, rather than a single circumpolar population, with the Bransfield Strait region notably exhibiting high genetic diversity (Galaska et al. 2017b). Despite having observed gene flow, the Antarctic sea star *Glabraster antarctica*, was found to have phylogeographic structuring, with genetic barriers evident between geographically proximate regions (Moore et al. 2018). Distinct genetic structure and cryptic speciation was found in the Antarctic crinoid *Promachocrinus kerguelensis* from samples collected along the Antarctic Peninsula and sub-Antarctic islands (Wilson et al. 2007). Supporting the hypothesis of glacial refugia in organisms with limited dispersal, evidence of strong genetic substructure was found in the brooding microbivalve *Kidderia subquadrate* (Levicoy et al. 2021). Strong genetic structure in the brooding bivalve *Lissaraca notorcadensis* was found between Antarctic and sub-Antarctic groups, with the evidence suggesting cryptic speciation in the species (Linse et al. 2007). Additionally, The Antarctic sponge *Dendrilla antarctica* has shown signals of population differentiation separating populations in the Central-southern WAP from the Bransfield Strait (Leiva et al. 2019).

Pycnogonids (sea spiders) are globally distributed arthropods that have a remarkable amount of recently diverged lineages (Mahon et al. 2008; Arango et al. 2011; Dietz et al. 2015; Ballesteros et al. 2021), are speciose in the Southern Ocean, and possess high rates of endemism (Aronson et al. 2007; Dömel et al. 2020). Studies examining population structure within Pycnogonida have shown distinct genetic differentiation within Antarctic species. Investigations into *Nymphon australe* with mitochondrial data (COI and 16S) have described it as a circumpolar species with high genetic diversity and limited gene flow. Distinct genetic differentiation among individuals from different localities and regions was found, exhibiting genetic evidence for phylogeographic structuring in the species (Mahon et al. 2008; Arango et al. 2011; Soler-Membrives et al. 2017). Mahon et al. (2008) found two unrecognized species of *Nymphon* that were morphologically identified as *Nymphon cf. australe,* unique haplotypes from six sampling locations of *N. australe,* and high levels of diversity within Nymphonidae. Arango et al. (2011) proposed that *N. australe* was a metapopulation compromised of a series of sub-divided populations that over time could result in speciation. Soler-Membrives et al. (2017) also found regionally distinct populations that may have undergone recent population expansion in *N. australe*. COI data revealed the pycnogonid *Colossendeis megalony*x to be a species complex consisting of about 15–20 distinct and unrecognized species (Dietz et al. 2015). Multiple phylogenetic analyses of COI sequences of *Pallenopsis patagonica* revealed that *P. patagonica* is a species-rich complex (Weis et al. 2014; Harder et al. 2016).

Nymphon australe is the most abundant and widely distributed species of sea spider in the Southern Ocean (Munilla and Membrives 2008; Mahon et al. 2008; Arango et al. 2011; Soler-Membrives et al. 2017). This species, like most other species of pycnogonids, is a paternal brooder, where fertilized eggs and subsequent larvae are carried by the paternal parent, resulting in an absence of a pelagic platonic life stage (Mahon et al. 2008). Its reproductive strategy combined with the slow ambulatory capabilities of adults suggests limited dispersal capabilities of *N. australe* (Poulin and Féral 1996; Mahon et al. 2008) and makes the documented circumpolar distribution in the Southern Ocean notable and worthy of investigation.

Utilizing advanced RAD-seq data to explore the circumpolar population structure of *N. australe* allows for a deeper understanding of evolutionary processes, genetic adaptations, and ecological dynamics in these enigmatic sea spiders. 3RAD is a method for preparing RAD-seq libraries that builds upon the strengths of double-digest restriction site-associated DNA sequencing (ddRAD-seq) while addressing the limitations of RAD-seq such as high up-front costs for adapters that are phosphorylated on both ends, which can form adapter dimers, the inability to reduce chimera formation, as well as the inability to multiplex high numbers of libraries, resulting in high sequencing costs (Bayona-Vásquez et al. 2019). The 3RAD method minimizes processing steps by ligating adapters in the presence of active restriction enzymes and includes an optional third restriction enzyme that cuts apart adapter-dimers formed by phosphorylated adapters, increasing the efficiency of adapter ligation to sample DNA. This method uses multiple PCR steps with additional primers that allow for fully active quadruple indexed illumina libraries that can be multiplexed, thus allowing pooling with any other illumina library type, and reducing the overall cost at buy in as well as per sample. These benefits make 3RAD a suitable choice for ecological research of non-model organisms such as *N. australe* (Kess et al. 2016; Bayona-Vásquez et al. 2019).

Our study aimed to examine genetic diversity, population connectivity, population differentiation and gene flow in the circumpolar *N. australe*. We recovered single nucleotide polymorphisms (SNPs) from 111 *N. australe* individuals

sampled from ten distinct geographic locations on the Antarctic continental shelf including the Western Antarctic Peninsula, Southern Atlantic Ocean, Ross Sea, Weddell Sea, and Eastern Antarctica. This investigation provides insight into the forces that impact the populations structure of *N. australe* in the Southern Ocean, and more broadly, the dynamics of the Antarctic benthos in the face of environmental and anthropogenic changes driven by climate change.

Materials and methods

Sample collection, preservation, and extraction

Specimens of *Nymphon australe* were collected via Blake trawls from ten distinct geographic locations including the Western Antarctic Peninsula, Southern Atlantic Ocean, Ross Sea, Weddell Sea, and Eastern Antarctica. Samples were collected over multiple research expeditions aboard the *RV/IB Nathaniel B. Palmer* (NBP 12–10, NBP 20–10, and NBP 23–03) and the *AS/RV Laurence M. Gould* (LMG 13–12). Upon collection, samples were flash frozen in liquid nitrogen and stored at – 80 °C or preserved in ~ 95% ethanol. Samples were transported to Central Michigan University and identified as *N. australe* Hodgson (1902) using morphological keys as well as the original description of the species (Child 1955, Hodgson 1902). A total of 111 individual *N. australe* were used in this study (Fig. 1, Table 1). All samples in this study are stored in the Mahon lab at Central Michigan University available upon reasonable request. DNA was extracted from each individual using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Inc.) according to the manufacturer's instructions.

3RAD library preparation, sequencing, and locus assembly

The 3RAD library preparation was performed following the modified ddRADseq protocol used in Peterson et al. (2012) provided by Matthew. R. Graham at Eastern Connecticut State University. Details of the full protocol used in this study are provided in Online Resource 1. Following the addition of sample-specific barcode tags, 3RADseq libraries were paired-end sequenced on a standard Illumina HiSeq X platform with 2 × 150 bp sequencing and synthesis (SBS) chemistry at a commercial sequencing facility (Azenta Life Sciences).

Resulting raw reads were demultiplexed by sample pool with unique illumina i5/i7. The sample pools were then further demultiplexed to obtain files with sequences specific to each individual inline barcodes using demultiplex v.1.2.2 (Laros 2023). Any reads that were not assigned by inline barcodes to individual samples were not retained for further analyses. Demultiplexed reads were then processed using the STACKS version 2.65 (Catchen et al. 2013). The STACKS *process_radtags* module was run on samples, where low-quality reads, reads with uncalled bases and reads without a complete barcode or restriction cut site were removed. This

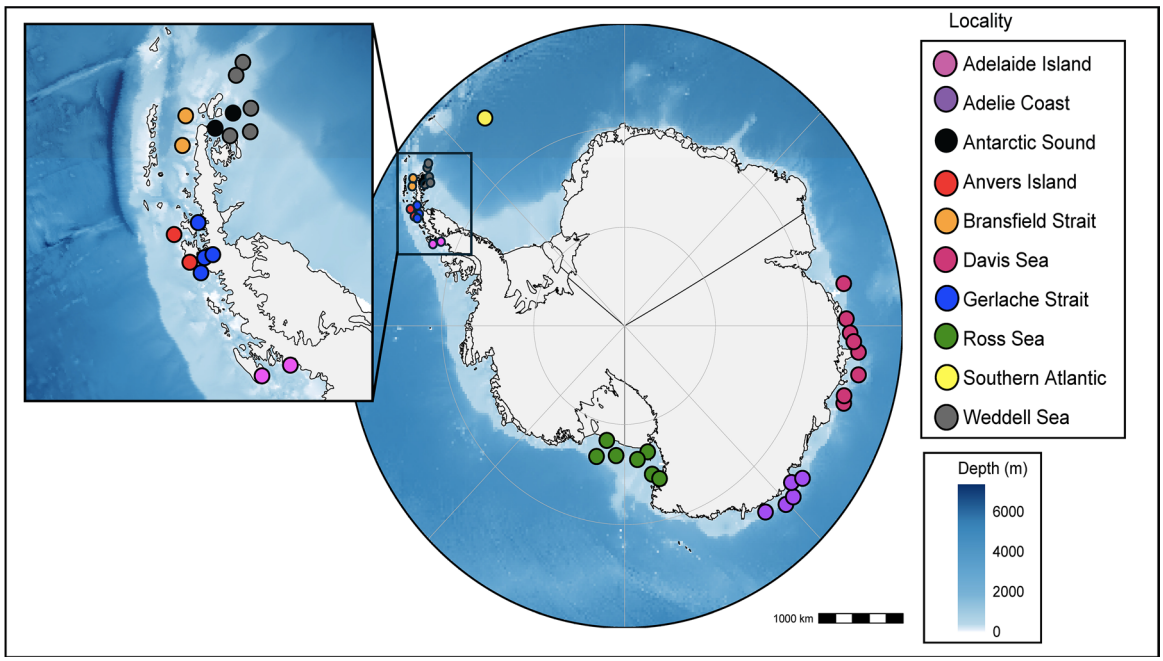

Fig. 1 Map of sampling locations of *Nymphon australe* used for this study

Table 1 Number of individuals from each sampling locality included in this study. Latitude and longitude of each sampling locality were also included

Location #	# of samples	Latitude	Longitude
Western Antarctic Peninsula-Adelaide Island			
Ch.125	4	− 67.52601	− 68.659221
Ch.649	2	− 68.238763	− 67.190652
Western Antarctic Peninsula-Anvers Island			
Ch.62	2	− 63.842200	− 62.640717
Ch.413	3	− 64.846183	− 62.959483
Western Antarctic Peninsula-Antarctic Sound			
Ch.325	2	− 63.685783	− 56.859000
Ch.333	2	− 63.753717	− 55.683983
Ch.587	3	− 64.104858	− 54.827107
Western Antarctic Peninsula-Bransfeild Strait			
Ch.316	4	− 64.035167	− 56.728250
Ch.625	3	− 63.057205	− 58.641628
Ch.629	3	− 62.71389	− 58.641628
Western Antarctic Peninsula-Gerlache Strait			
Ch.290	1	− 64.411200,	− 61.963167
Ch.395	4	− 65.099833	− 63.165667
Ch.397	5	− 65.020867	− 64.425033
Ch.633	5	− 64.96022	− 63.52269
Western Antarctic Peninsula-Weddell Sea			
Ch.509	4	− 63.333687	− 53.212952
Ch.533	1	− 63.333687	− 53.212952
Ch.553	1	− 63.333687	− 53.212952
Ch.561	1	− 64.436367	− 55.832383
Ch.574	3	− 64.436367	− 55.832383
Southern Atlantic Ocean			
Ch.322	4	− 64.134392	− 36.860217
Ross Sea			
Ch.243	2	− 76.479247	− 165.7441
Ch.246	3	− 78.040705	− 169.9307
Ch.251	1	− 76.998275	− 175.0932
Ch.256	2	− 76.998275	− 175.093200
Ch.259	2	− 76.245262	174.50412
Ch.265	4	− 76.903800	169.96525
Ch.270	2	− 74.70781	168.407827
Ch.277	3	− 74.181977	166.661027
Eastern Antarctica-Adelie Coast			
Ch.700	2	− 65.895882	140.712585
Ch.714	2	− 64.992547	135.682252
Ch.720	5	− 64.992547	135.682252
Ch.736	5	− 65.081062	133.869843
Ch.744	5	− 65.081062	133.869843
Eastern Antarctica-Davis Sea			
Ch.751	2	− 65.059188	108.083948
Ch.759	1	− 65.059188	108.083948
Ch.772	1	− 65.204652	106.682137
Ch.788	1	− 64.45037	101.070275
Ch.794	3	− 64.555192	96.001992
Ch.798	3	− 65.695282	91.630302
Ch.806	4	− 65.954900	88.595117
Ch.819	1	− 66.030813	80.016687

was followed by the *denovo_map.pl* pipeline being run on the remaining reads. The STACKS *populations* module with the '--write-single-SNP' option was run on the data to limit the potential of linkage disequilibrium as downstream analysis require independent loci. A series of filtering schemes was evaluated, and the final dataset was selected for the amount of SNP coverage across genetically diverse individuals. Loci had to occur in 40% of individuals within a sampling locality (− r 0.4) and had to be present in at least one of the localities to be retained (− p 1). The minimum minor allele frequency required to process a nucleotide site at a locus was set at 0.05 (--min-maf 0.05), and the maximum observed heterozygosity required to process a nucleotide site at a locus was set at 0.5 (--max-obs-het 0.5).

Principal component analyses (PCAs) were performed by locality and by individual using the Analysis of Ecological Data: Exploratory and Euclidean Methods in Environmental Sciences (*ade4*) v1.7–22 package (Dray and Dufour 2007) in *R* v4.3.1 (R Core Team 2022). The top three principal components were iteratively compared. Samples were further analyzed using the Discriminant Analysis of Principal Components (DAPC) in the *adegenet* v2.1.10 package in *R* v4.3.1 (Jombart 2008; Jombart and Ahmed 2011) to determine group (population) membership across localities by conducting a series of PCAs on the dataset and then performing a Discriminant Analysis on the retained principal components. The optimal number of clusters (K) was determined through Bayesian information criterion (BIC) likelihood values from the retained principal components.

Population structure and potential admixture was assessed using the Landscape and Ecological Associations (*LEA*) v2.0 package in R (Frichot and François 2015). Individual admixture coefficients were estimated by using the R function snmf on the data with 100 repetitions, the K set at 1:10, and entropy set to true. The estimation of K (number of ancestral populations) in *LEA* was determined using the minimum cross-entropy criterion and least-squares estimates.

The HIERFSTAT v. 0.5–11(Goudet 2005) package in R was used to generate summary statistics and estimate genetic differentiation for the samples from each of the ten locations. These statistics included the private alleles at each location, expected and observed heterozygosity (Ho and Ht), as well as the inbreeding coefficient (*Fis*). Pairwise genetic distance (*Fst*) for each locality was also estimated (Nei 1987; Goudet 2005). Lastly, to investigate potential migration patterns between locations included in this study, the *divMigrate* function in the R package *DiveRsity* v.1.9.90 (Keenan et al. 2013) was used to calculate directional migration rates between each location.

Results

A total of 139,195 single nucleotide polymorphic (SNP) loci were recovered from *Nymphon australe* individuals included in the dataset. After quality filtering (see above), 18,566 independent SNP loci were retained for downstream analysis.

The principal components analysis (PCA) by locality indicates that there is genetic structure found between all the localities (Fig. 2). Specifically, the PCA revealed that the Bransfield Strait, Adelaide Island, and Davis Sea locations have a large amount of genetic variation (Fig. 2).

Supporting results observed in the PCA, the DAPC analyses recovered three clusters within *N. australe* (K = 3) with three regional populations with some genetic overlap occurring (Fig. 3). The first cluster contained individuals primarily from the Weddell Sea, Southern Atlantic, Bransfield

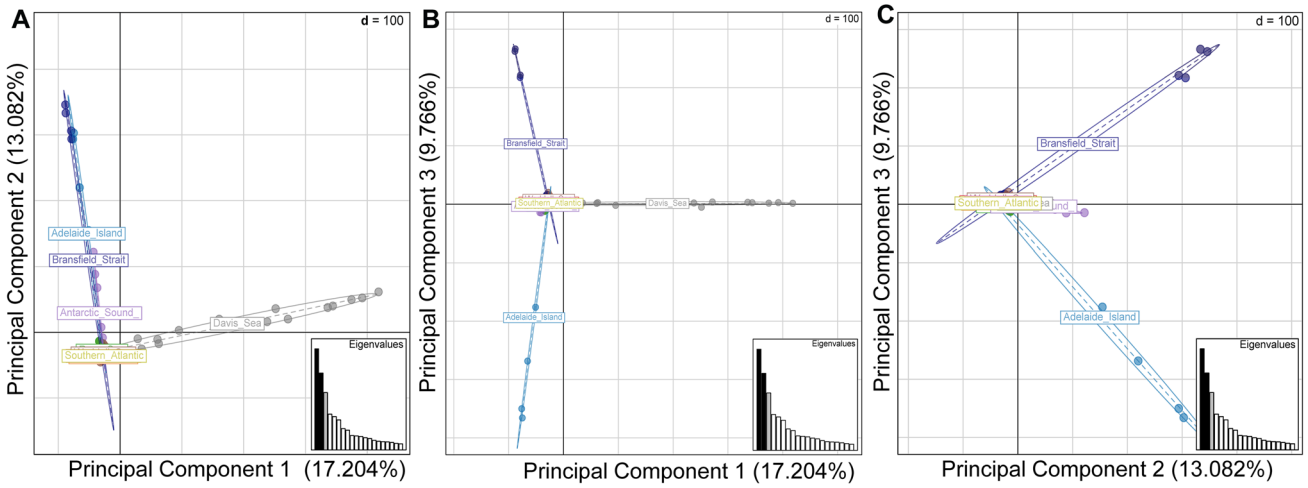

Fig. 2 Principal components analysis by collection locality comparing the top three principal components

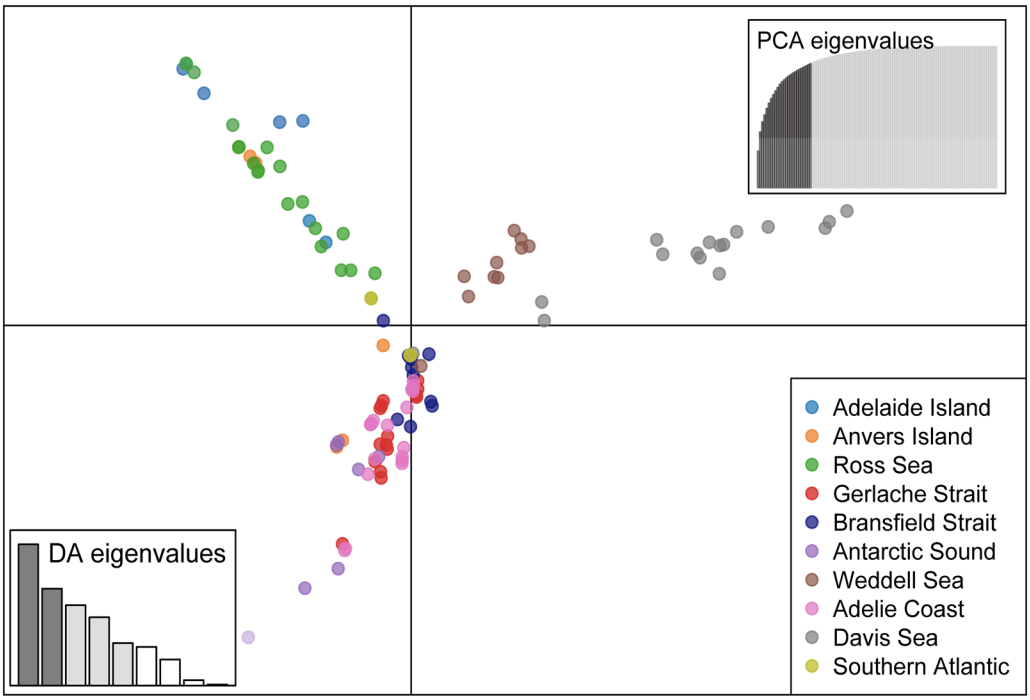

Fig. 3 Discriminant Analysis of Principal Components for *Nymphon australe* single nucleotide polymorphism data

Strait, Adelie Coast, and Antarctic Sound localities (Fig. 3). The second genetic cluster contained individuals primarily from the Adelaide Island and Ross Sea localities (Fig. 3). The third cluster included only individuals from the Davis Island locality (Fig. 3).

Cross-entropy and admixture analyses in LEA recovered finer scale phylogeographic structure than the PCA and DAPC analyses, with seven ancestral populations of *N. australe* having the highest statistical support (K = 7, Fig. 4). Due to the small decrease in cross-entropy of K from 4 to 5 (Fig. 4), K = 4 was also explored (Online Resource 2), to ensure there was no difference in interpretation of ancestral admixture or any clearer inferences to be drawn. Admixture was present across all localities, but patterns of distinct geographic structure were recovered (Fig. 4). The first ancestral genotype was predominant in individuals from the Ross Sea, while the second ancestral genotype prevailed in those from the Bransfield Strait, Antarctic Sound, and Adelaide Island (Fig. 4). Individuals from the Weddell Sea predominantly exhibited the third ancestral genotype. The fourth ancestral genotype was present in individuals from all localities except the Weddell Sea, and notably, it constituted a majority of the genotype in a single individual from the Davis Sea. Similarly, the fifth ancestral genotype was mainly found in individuals from the Bransfield Strait, Adelaide Island, and Antarctic Sound. The sixth ancestral genotype was common among individuals from the Weddell Sea and Bransfield Strait, and it was also one of the most abundant genotypes in the Adelie Coast, Ross Sea, Anvers Island, and Gerlache Strait. Finally, the seventh ancestral genotype predominated in individuals from the Davis Sea and was also abundant in individuals from Anvers Island, the Southern Atlantic, Ross Sea, Bransfield Strait, and Gerlache Straight (Fig. 4). Notably, the localities with the highest proportion of admixed individuals were the Adelie Coast, Ross Sea, and Gerlache Strait (Fig. 4).

Each *N. australe* collection locality contained private alleles (Table 2). The Davis Sea locality contained the most private alleles, and the Adelie Coast locality contained the least number of private alleles (691 and 13, respectively). The observed heterozygosity was lower than the expected heterozygosity for all localities (Table 2). Inbreeding coefficients were positive for all putative populations, with the largest inbreeding coefficient observed in the Antarctic Sound locality (0.9720) and the lowest observed inbreeding coefficient in the Bransfield Strait locality (0.8527) (Table 2). The Bransfield Strait and Southern Atlantic localities had the lowest pairwise genetic distance observed (0.124) (Fig. 5). The Adelaide Island and Adelie Coast localities had the highest observed pairwise genetic distance (0.545) (Fig. 5). A hierarchical analysis of molecular variance (AMOVA) of the SNP data gave statistical significance to the grouping of populations according to their geographical location (Online Resource 3).

Relative directional migration rates between the ten localities are shown as a network in Fig. 6. Arrows indicate the

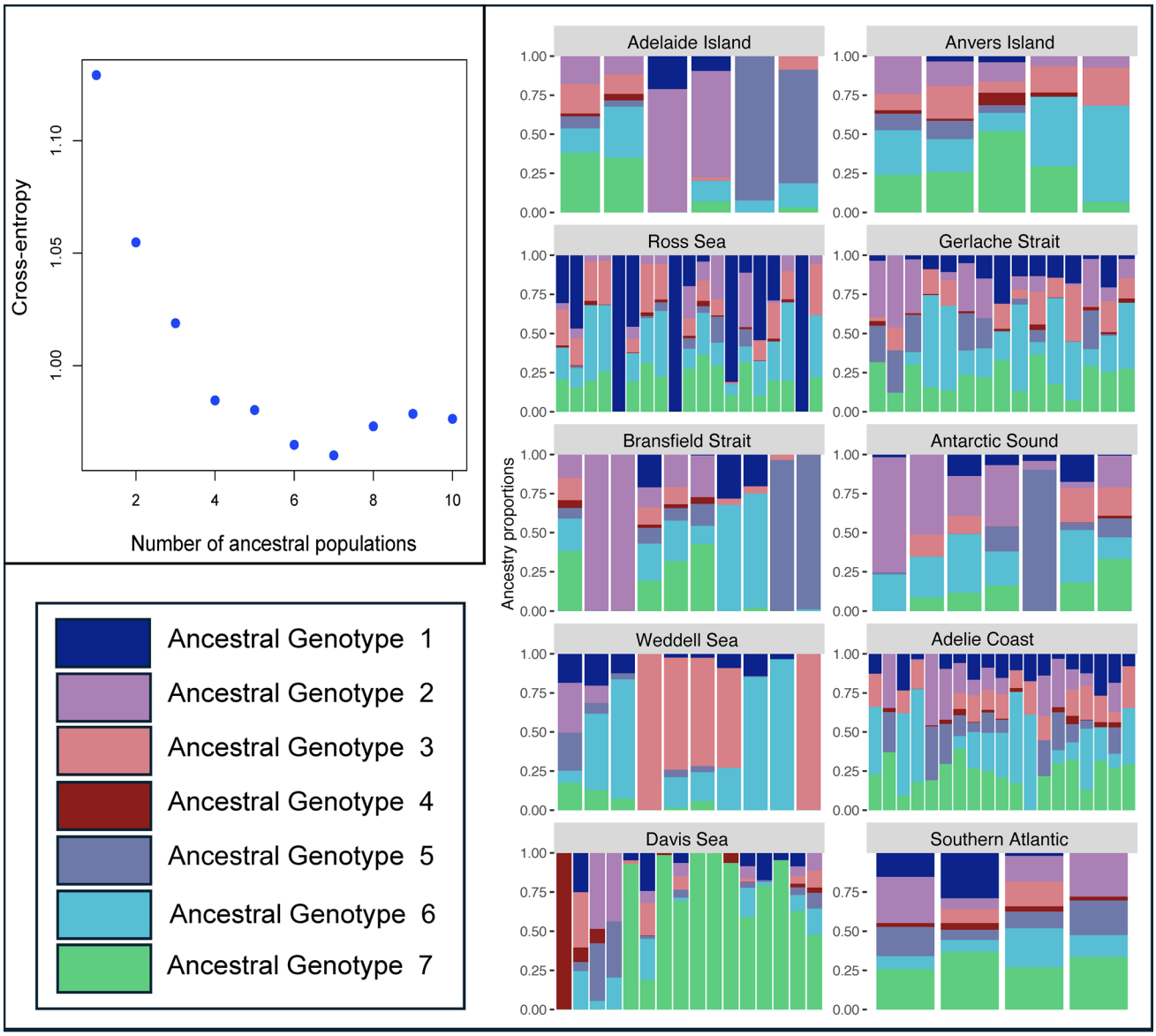

Fig. 4 Cross-entropy plot, and ancestry matrices of individuals within each locality for *Nymphon australe* in this study

Table 2 Summary statistics for each of the localities for *N. australe*

Locality	Private alleles	*Ho*	*Ht*	*Fis*
Adelaide Island	447	0.0292	0.2162	0.8650
Adelie Coast	13	0.008	0.0161	0.9476
Antarctic Sound	310	0.002	0.0063	0.9720
Anvers Island	36	0.009	0.309	0.9701
Bransfield Strait	311	0.0264	0.1792	0.8527
Davis Sea	691	0.0219	0.2314	0.9053
Gerlache Strait	16	0.0010	0.0211	0.9511
Ross Sea	199	0.0016	0.0468	0.9650
Southern Atlantic	29	0.0002	0.0063	0.9720
Weddell Sea	194	0.0051	0.0829	0.9383
Overall	2246	0.0105	0.1360	0.8923

recovered direction of gene flow, and the relative strength based on the bootstrap support values. The highest level of gene flow was observed going from the Adelie Coast to the Gerlache Strait (Nm = 1) (Fig. 6). There is observed gene flow to the Bransfield Strait locality with gene flow from the Ross Sea (Nm = 0.9), Adelie Coast (Nm = 0.51), Adelaide Island (0.42) and Gerlache Strait (Nm = 0.27) localities. The Adelie Coast locality also showed low to relative levels of gene flow from the Weddell Sea (Nm = 0.50), and Anvers Island (Nm = 0.33) localities. The Anvers Island locality was observed to have small amounts of gene flow from the other localities. The Antarctic Sound locality showed a small amount of relative gene flow only to and from the Adelaide Island locality (Nm = 0.02) (Fig. 6). The Davis Sea and

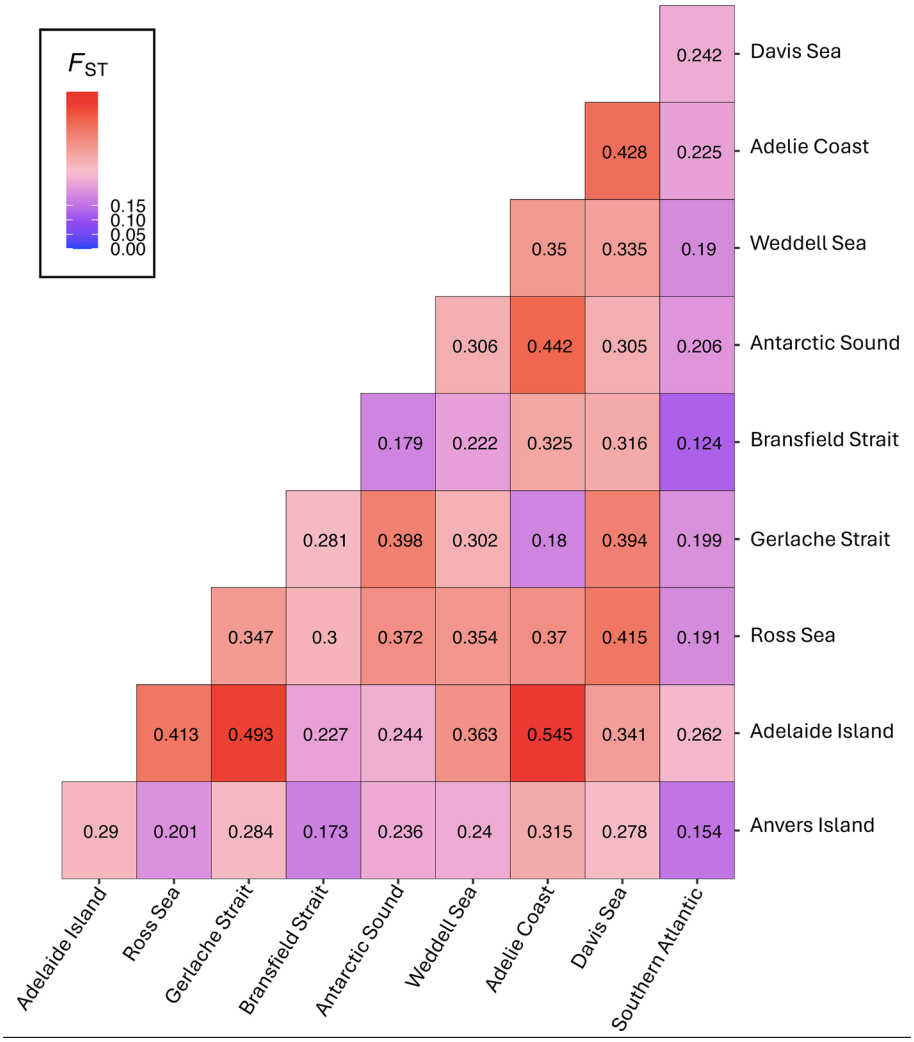

Fig. 5 Pairwise genetic distances (Fst) displayed as a heatmap for the putative *Nymphon australe* populations

Southern Atlantic localities did not recover any gene flow to or from any of the other localities (Fig. 6).

Discussion

Genomic analysis revealed significant geographic structure for *Nymphon australe*. DAPC identified three regional populations and LEA admixture analysis revealed finer-scale population structure. This geographic structuring likely reflects limited gene flow between populations caused by geographic barriers, glacial refugia, reproductive strategies, and the limited dispersal capabilities of *N. australe*.

The DAPC and PCA results revealed a cluster primarily comprising individuals from the Weddell Sea, Southern Atlantic, Bransfield Strait, Adelie Coast, and Antarctic Sound localities, with the Bransfield Strait likely acting as a central hub due to the mixing of distinct water masses in the area (Gordon and Nowlin Jr 1978; Hoffman et al. 2012). The Antarctic Circumpolar Current (ACC) contributes to eastward distribution from localities in the WAP, while the clockwise movement of the Weddell Sea Gyre likely facilitating intermixing with populations from the Weddell Sea, Antarctic Sound, and Southern Atlantic. These connections are supported by lower *Fst* values observed between localities, relative migration rates into the Bransfield Straight locality, and similar patterns observed in other Antarctic benthic invertebrates (Wilson et al. 2007; Hoffman et al. 2012; Dueñas et al. 2016; Galaska et al. 2017a; Moore et al. 2018; Leiva et al. 2018, 2019; Levicoy et al. 2021).

Notably, Adelaide Island and Ross Sea samples also clustered in a separate population. This is possibly due to the existence of semi-isolated gyres resulting from the interaction between the Ross Sea Gyre, Antarctic Circumpolar Current (ACC), and Antarctic Coastal Current (ACoC) (Muñoz-Ramírez et al. 2020). These potential gyres may hinder the northward movement of Ross Sea populations beyond Adelaide Island, supported by previous studies on the possible presence of semi-isolated gyres in the region and fine-scale genetic differentiation in Antarctic limpets (Hofmann et al.

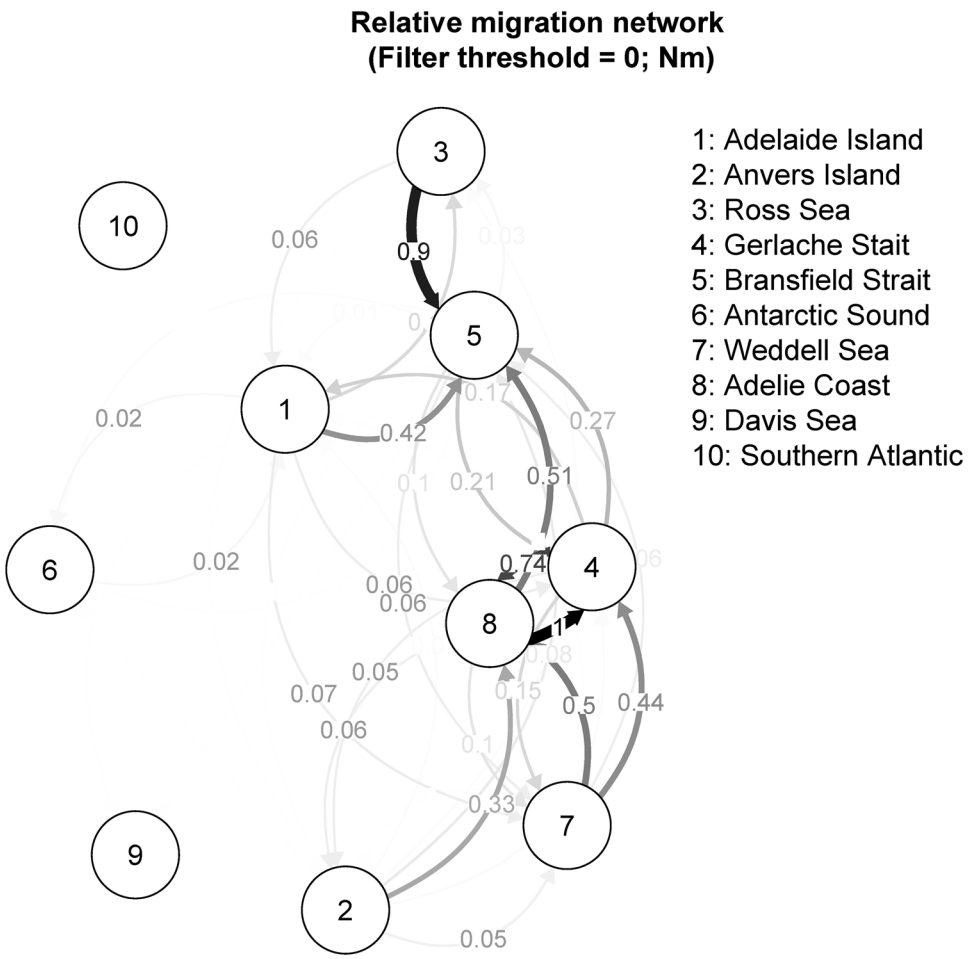

Fig. 6 Relative migration network of *N. australe* putative populations, assessing each Antarctic sampling locality, with the shading of the lines indicate the strength of the connection between the localities

1996; Hoffman et al. 2012). Alternatively, the existence of a trans-West Antarctic seaways connecting the Weddell Sea, Amundsen Sea and Ross Sea in the past, may explain some of the similarity between the Ross Sea and eastern Antarctic Peninsula samples (Strugnell et al. 2018; Lau et al. 2023). However, more samples from the Weddell Sea region would be needed to fully consider this hypothesis.

The Davis Sea population was recovered as a separate cluster from the rest of the sampled populations, had the highest number of private alleles, and showed more genetic similarity to the Weddell Sea population than the Adelie Coast sites. The Antarctic Slope Current (ASC) exhibits varying intensity across Antarctica, becoming surface-intensified in areas with lighter shelf water and denser offshore waters, while becoming bottom-intensified in regions with intense sea ice formation, affecting the connection between the continental shelf and open ocean (Huneke et al. 2023). The Davis Sea samples were collected from Eastern Antarctica with a surface-intensified ASC, contrasting with the bottom-intensified ASC in other Antarctic regions (Huneke et al. 2023), potentially affecting the distribution and dispersal of *N. australe* in the region.

Despite differences in dispersal capability and life stages, the population structure found in *N. australe* is strikingly similar to what has been observed in other Antarctic invertebrates (Wilson et al. 2007; Hunter and Halanych 2008; Hoffman et al. 2012; Galaska et al. 2017a,b; Moore et al. 2018; Leiva et al. 2018, 2019; Levicoy et al. 2021). For example, the crinoid *P. kerguelensis* has a documented wide distribution and high dispersal capabilities but was found to have high genetic structure and cryptic speciation from collection locations along the Peninsula and sub-Antarctic islands (Wilson et al. 2007). Patterns of genetic differentiation were observed in *Dendrilla antarctica*, a species of sponge that has lecithotrophic larvae (Leiva et al. 2019). The shared patterns of genetic structure suggest fundamental processes such as ocean currents, gyres, sea ice dynamics, shelf water properties, species-specific traits, and historical factors play a role in the Antarctic benthos. These comparable findings of genetic structure emphasize the importance of understanding the ecosystem and population dynamics in this region.

Additionally, the role of oceanic currents closer to the Antarctic continent, (i.e. the Weddell Gyre, Ross Gyre and ACoC) may help define regional variability in the Southern

Ocean. These systems form oceanographic and ecological systems that are different from those affected by the ACC (Gäbler-Schwarz et al. 2021). These ecosystems are subject to different environmental factors, such as the seasonality of sea ice coverage (Stammerjohn et al. 2012), which can lead to differences in temperature, salinity and nutrient availability, and may impose diverse selective pressures on the resulting populations, thus driving local adaptations and speciation.

Differences in the number of populations (K) recovered by both programs can be attributed to their distinct underlying models and assumptions. DAPC tends to be less conservative in detecting population structure as it prioritizes maximizing between-group variance and minimizing within-group variance without explicitly modeling population (Jombart 2008; Jombart and Ahmed 2011). LEA provides more conservative estimates due to its use of latent factor mixed models that explicitly model population structure based on allele frequencies, offering detailed insights into complex population patterns such as hierarchical and continuous structure (Frichot et al. 2014; Frichot and François 2015).

The reproductive biology of *N. australe* offers a likely explanation for the significant phylogeographic structuring, the lower observed than expected heterozygosity and high inbreeding coefficients observed in this study. Species with brooding life histories are presumed to have low dispersal capabilities and reduced gene flow, and show strong spatial genetic structure in their populations (Thatje et al. 2005; Mahon et al. 2008; Boissin et al. 2015). Although this study found clear genetic differentiation by location, the observed gene flow between populations could be explained by rafting on substrates or other organisms such as soft coral or jellyfish (Fraser et al. 2010; Leese et al. 2010; Thatje 2012; Bartlow and Agosta 2021).

The findings of this study support the conclusion by Arango et al. (2011) that *N. australe* is most likely a metapopulation comprised of a series of sub-divided populations. Concurrently, these results also support the conclusions by Mahon et al. (2008) and Soler-Membrives et al. (2017) that *N. australe* shows high genetic diversity, and may be comprised of regionally distinct populations that may have undergone recent population expansion. Similar to Mahon et al. (2008), the specimens included this study were morphologically indistinguishable from one another, yet genetically distinct, indicating that *N. australe* may also be a species complex in the Southern Ocean, as seen in other population genomic studies within Pycnogonida (Weis et al. 2014; Dietz et al. 2015; Harder et al. 2016). Further investigations examining possible correlations between morphologically variable traits (e.g., the number chelifore teeth or number of ovigerous spines and genetic variation) are needed to further determine whether *N. australe* is a species complex.

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Author contributions JRZ contributed to the sample collection, conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing the original draft as well as a review and editing. MPG contributed to the data analysis, investigation, visualization as well as the review and editing of the manuscript. KMH contributed to funding acquisition, sample collection, investigation as well as review and editing of the manuscript. ARM contributed to funding acquisition, conceptualization, project administration, sample collection, resources, supervision, investigation, validation as well as review and editing of the manuscript.

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Data availability Online Resources and raw reads for 3RAD restriction site-associated DNA single nucleotide polymorphism data are deposited in Dryad (doi:10.5061/dryad.1vhhmgr28). (private link for reviewers: https://datadryad.org/stash/share/JP1c2Mx_6bWx1zeCjEekh_KFT_7uBExg6Yu5oDcjNM0).

Declarations

Conflict of interest Authors declare no competing interests or conflict of interest exist.

Ethical approval No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Informed consent No humans formed the basis of this study; therefore, no informed consent was required.

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