Population Genomics of *Nymphon australe* Hodgson, 1902 (Pycnogonida, Nymphonidae) in the Western Antarctic

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Abstract. Within the Southern Ocean, the Antarctic Circumpolar Current is hypothesized to facilitate a circumpolar distribution for many taxa, even though some, such as pycnogonids, are assumed to have limited ability to disperse, based on brooding life histories and adult ambulatory capabilities. With a number of contradictions to circumpolarity reported in the literature for other pycnogonids, alternative hypotheses have been explored, particularly for *Nymphon australe*, the most common species of Pycnogonida (sea spider) in the Southern Ocean. Glacial events have been hypothesized to impact the capacity of organisms to colonize suitable areas without ice coverage as refuge and without the eurybathic capacity to colonize deeper areas. In this study, we examine populations of one presumed circumpolar species, the pycnogonid *N. australe*, from throughout the Western Antarctic, using a 2b-RAD approach to detect genetic variation with single-nucleotide polymorphisms. Using this approach, we found that *N. australe* included two distinct groups from within >5000-km sampling region. By using a discriminant analysis of principle components, sparse nonnegative matrix factorization, and admixture coefficient analysis, two distinctive populations were revealed in the Western Antarctic: one covered distances greater than 5000 km (Weddell, Western Antarctic Peninsula, and Ross Sea), and the other shared limited connectivity entrained within the Amundsen Sea. Under further scrutiny of the 3086 single-nucleotide polymorphisms in the data set, only 78 loci had alignment stacks between the two populations. We propose that the populations analyzed are divergent enough to constitute two different species from within this common Antarctic genus known for its phenotypic plasticity.

Introduction

The benthic communities of the Southern Ocean are vastly different from those found in other regions of the world because of the ocean's geological and thermal isolation (Peck *et al.*, 2006). The onset of the Antarctic Polar Front (APF) about 41 million years ago (mya) isolated the region, including the benthic fauna, through a number of geological processes (Scher and Martin, 2006). The APF is postulated to be responsible for isolating Southern Ocean biota and for the high rates of endemism for organisms unable to cross the differential in temperature and salinity (Clarke *et al.*, 2005; Thornhill *et al.*, 2008; Kaiser *et al.*, 2013). Although oceanic fronts have the potential to act as barriers to dispersal, oceanic currents also have the potential to act as dispersal vectors (Scheltema, 1986; Clarke *et al.*, 2005; Thornhill *et al.*, 2008; Galaska *et al.*, 2017a). The Antarctic Circumpolar Current (ACC) has long been a force credited with the dissemination of larval and/or adult life stages, resulting in homogeneous populations around the continent of Antarctica (Arntz *et al.*, 1994; Clarke and Johnston, 2003). Along with the ACC, coastal currents, Antarctic deep-water currents, the Ross Sea Gyre, and the Weddell Sea Gyre, for example, have a significant

impact on organisms inhabiting the Antarctic Shelf (Riesgo *et al.*, 2015).

Antarctic glaciation was initiated during the Cenozoic when atmospheric CO_2 decreased (DeConto and Pollard, 2003). Glacial activity limited the availability of habitable space on the Antarctic Shelf (Thatje *et al.*, 2005). Glacial coverage is not static, rendering habitat necessary for the survival of benthic organisms unreliable over a geologic timescale (Berger, 1988; Thatje *et al.*, 2005). For this reason, benthic organisms have had potential paths to persist through periods of glacial maxima. Eurybathic species could seek refuge in the depths or within deglaciated areas (Thatje *et al.*, 2005). During periods of high glacial cover, subsets of organismal populations become isolated from one another, whereas times of glacial minima allow for the mixing of populations that are no longer separated (Thatje *et al.*, 2005). Alternating periods of isolation and coalescence of taxa under fluctuating environmental conditions led to the condition dubbed the "diversity pump" (Clarke and Crame, 1992). The diversity pump functions at two scales: long-term climatic fluctuations influence the success of particular physiologies and ecologies for organisms, and shorter cycles influence their distribution (Clarke and Crame, 1992).

Pycnogonids (sea spiders) are globally distributed marine arthropods and are a ubiquitous component of the Southern Ocean benthic community. Sea spiders in the Southern Ocean are speciose, with more than 192 described species, 108 of which are endemic to the region (Munilla and Soler-Membrives, 2009). The brooding reproductive strategies of pycnogonids and the particularly slow ambulatory capabilities of adults would suggest limited dispersal capabilities (Poulin and Feral, 1996). However, many species are reported to be circumpolar, based simply on presence/absence data (Munilla and Soler-Membrives, 2009). Recently, some pycnogonid species previously thought to have circumpolar distributions have been shown to be a cryptic species complex (Krabbe *et al.*, 2010; Weis *et al.*, 2014; Dömel *et al.*, 2017). *Nymphon australe* Hodgson, 1902 is the most commonly found species of pycnogonids in the Southern Ocean, and it has, as do nearly all pycnogonids, a brooded, lecithotrophic larval protonymphon stage (Arango *et al.*, 2011; Brenneis *et al.*, 2017). The species is noted for high phenotypic plasticity, and previous studies also found high genetic diversity within the species (Child and Cairns, 1995; Mahon *et al.*, 2008; Arango *et al.*, 2011; Soler-Membrives *et al.*, 2017). For example, phenotypically variable or plastic traits include the number of chelae teeth per finger (36–65), a short or long propodus, and vestigial or absent auxiliary claws; but every character can vary, even in individuals sampled from the same trawl (Child and Cairns, 1995).

Most recently, Soler-Membrives *et al.* (2017) found *N. australe* to have a circumpolar distribution and geographically structured haplotypes by using mitochondrial DNA sequences from a partial fragment of cytochrome c oxidase subunit I (*COI*).

High phenotypic plasticity, as well as the structured nature of the *COI* data, necessitates utilization of a high-resolution genomic technique and an increased sampling effort to accurately assess the diversity and population connectivity of *N. australe* in the Southern Ocean. A single uniparentally (maternally) inherited marker does not provide enough resolution to detect *N. australe* admixture of populations. Further, Soler-Membrives *et al.* (2017) did not use *16S* and internal transcribed spacer ribosomal RNA fragments or microsatellite markers because they did not show sufficient sequence variation. The use of restriction site-associated DNA sequencing (RADseq) to discover single nucleotide polymorphisms (SNPs) and to genotype nonmodel organisms is cost effective, easily generates hundreds to thousands of SNPs, and provides the ability to differentiate populations with confidence (Andrews *et al.*, 2016). With the greatly improved resolution generated by our RADseq analyses, we hypothesize that we can distinguish between one homogenous population, geographically distinct populations, or cryptic species of *N. australe* throughout the Western Antarctic, as potential explanations for the observed pattern of circumpolarity of one *N. australe* species.

Materials and Methods

Sample collection

Specimens of *Nymphon australe* Hodgson, 1902 were collected *via* Blake trawls during two research expeditions aboard the RVIB *Nathaniel B. Palmer* (NBP12-10) and on the ASRV *Laurence M. Gould* (LMG13-12). Sampling sites included locations in the Ross, Bellingshausen, Amundsen, and western Weddell Seas and along the Western Antarctic Peninsula, covering an over-water distance of more than 5000 km (Fig. 1). Upon collection, specimens were sorted to morphospecies and preserved either at $-80\,°C$ or in ~95% ethanol. Subsequent identification to species was done upon return of samples to our home institution. For this investigation, a total of 92 individuals were included from throughout the sampled range, including 39 from the Western Antarctic Peninsula, 2 from the Bellingshausen Sea, 23 from the Amundsen Sea, and 28 from the Ross Sea (Fig. 1; Table A1).

Molecular methods

Genomic DNA extractions were performed using the QIAGEN (Hilden, Germany) DNeasy Blood and Tissue Kit, following the manufacturer's protocol. The 2b-RAD protocol was utilized because it uses type IIB restriction enzymes, which cleave the DNA into short, uniform fragments, allowing for even sequencing coverage across the genome (Wang *et al.*, 2012). With 2b-RAD, a subset of restriction sites can be targeted by using modified oligonucleotide adaptors that reduce genome complexity, thus decreasing marker density and allowing more individuals to be multiplexed per lane (Wang *et al.*, 2012). Samples were prepped using the 2b-RAD protocol (Wang *et al.*, 2012), with the *Alf1* restriction enzyme. A 1/8 re-
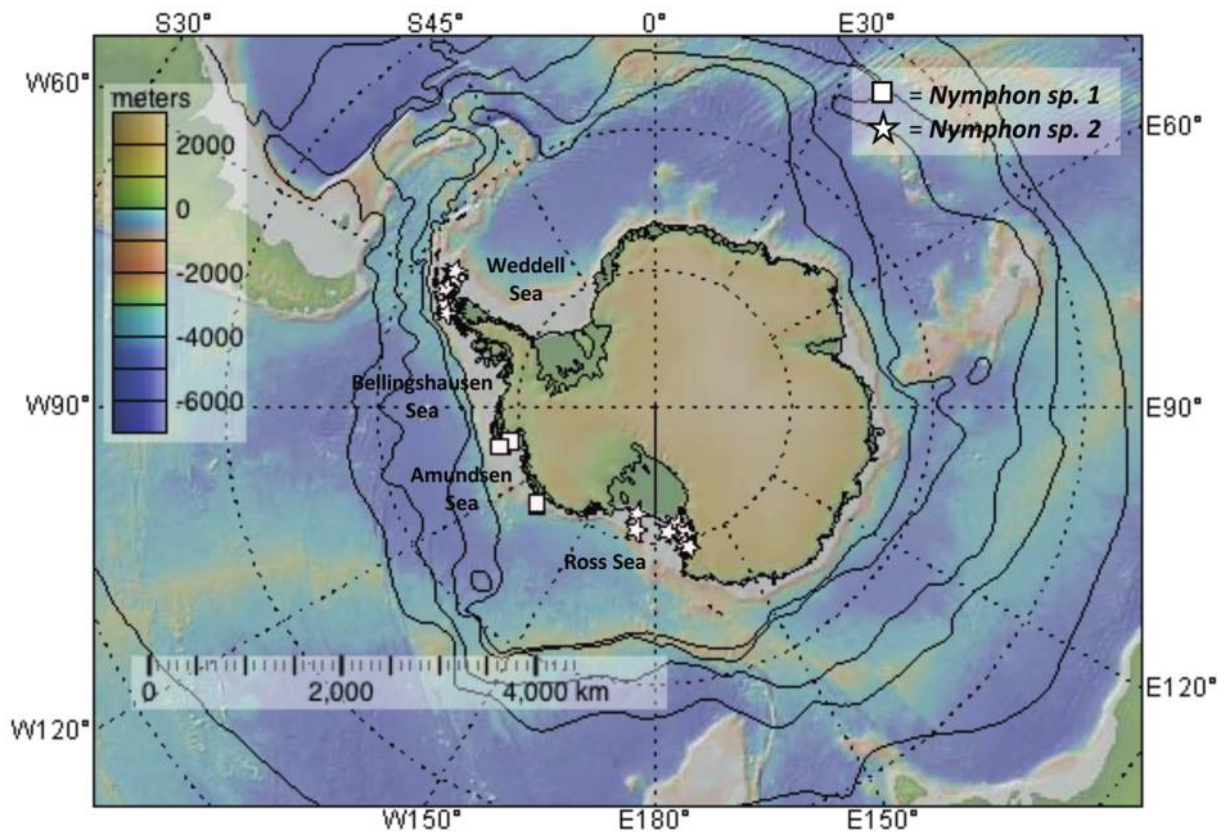
Figure 1. Samples were collected from the Antarctic Peninsula, Bellingshausen Sea, Amundsen Sea, and Ross Sea. Individuals retained after single-nucleotide loci filtering are referred to as *Nymphon* sp. 1 and 2. *Nymphon* sp. 1 is represented by squares, and the 12 individuals retained are located in the Amundsen Sea. *Nymphon* sp. 2 is represented by stars, and the 49 individuals retained are located surrounding the Antarctic Peninsula and the Ross Sea. Southern Ocean bathymetry is represented in meters, with the color scale on the left. This map was created using the GeoMappApp, version 3.6.4, tool (Ryan *et al.*, 2009).

duction scheme was selected on the basis of the estimated genome size of about 500 megabases (Mb). Approximate genome size was determined from previously sized sea spider genomes ranging between 205 and 743 Mb (Libertini and Krapp, 2007). The 1/8 reduction scheme was accomplished with the addition of adaptors 5ILL-NC and 3ILL-NG. Immediately following the 2b-RAD protocol, gel purification was performed using a QIA-GEN QIAquick Gel Extraction Kit. All samples had been incorporated with unique barcode combinations during the protocol and were pooled in equal concentrations prior to sequencing (Wang *et al.*, 2012). The prepared samples were then sequenced at the HudsonAlpha Institute for Biotechnology (Huntsville, Alabama) on an Illumina (San Diego, CA) HiSeq 2500 platform, using v4 chemistry to generate 50-bp single-end reads.

Data analyses

Raw sequence reads were de-multiplexed by individual, on the basis of unique barcode combinations used in 2b-RAD

preparation. Resulting FASTQ files (Illumina) were filtered to remove any samples that did not contain the *Alf* 1 restriction enzyme and were subsequently truncated to the uniform 36-bp amplicon with the AlfIExtract.pl 2.0 script from Meyer (2016). Loci were removed from further analyses if they did not have ≥15X coverage, <1% variance if homozygous, >25% variance if heterozygous, presence in ≥10% sample sites (collection latitude and longitude; Table A1), and ≥70% of individuals within sample sites, with the denovo_map.pl 2.0 script and populations program from *Stacks* (Catchen *et al.*, 2011, 2013).

The data set was evaluated as follows under the assumption that all *N. australe* individuals collected were one species. To estimate the most likely number of populations (K), we used the adegenet, version 2.0.1, package (Jombart, 2008; Jombart and Ahmed, 2011) in R 3.3.2 (R Core Team, 2016). Adegenet estimates K, or the presumed number of populations, by evaluating Bayesian information criterion values informed by retained principal components. Principal Component Analyses

(PCAs) are useful to spatially compare allele frequencies of loci among all individuals. The retained PCAs were used to perform a discriminant analysis of principle components (DAPC) within adegenet. The DAPC recovers maximum genetic variation between clusters, while minimizing genetic variation within clusters (Jombart, 2008; Jombart and Ahmed, 2011).

The Landscape and Ecological Associations 1.6.0 (LEA) package (Frichot and François, 2015) in R implements sparse nonnegative matrix factorization least squares optimizations to estimate population structure and visualize the results (Frichot *et al.*, 2014). Estimation of K is evaluated using the minimum cross-entropy criterion. Once K is determined, admixture coefficient analyses were plotted in LEA to assess the genetic mixing of populations.

The summary statistics, Mantel test, and pairwise fixation index (F_{ST}) values were calculated with samples separated into geographic regions (localities in Table A1; Wier and Cockerham, 1984). Summary statistics of SNP loci were calculated in HIERFSTAT, version 0.4.22 (Goudet, 2005). Mantel tests were performed to compare distance between geographic regions and pairwise F_{ST} values to test for isolation by distance. Pairwise F_{ST} values were calculated in HIERFSTAT, and distances between geographic regions were measured with the ruler tool in Google Earth Pro (Google, 2017). One distance was measured between the east coast of the Antarctic Peninsula and the Ross Sea by going around the continent as the ACC travels, and a second distance was measured over land between the east coast of the Antarctic Peninsula and the Ross Sea to represent the distance for a trans-Antarctic seaway (Barnes and Hillenbrand, 2010). Genetic differentiation was assessed for all individuals, and then pairwise comparisons were made on the basis of geographic regions.

Results

After application of final filtering steps to raw sequencing reads, 61 of the initial 92 individuals remained in our data set, and 3086 SNP loci were retained. Individuals retained after SNP loci filtering collected from locations surrounding the Antarctic Peninsula numbered 23; in the Ross Sea, 26; and in the Amundsen Sea, 12. Individuals were removed from the data set if they no longer retained informative loci after filtering. Raw sequencing reads were deposited in the National Center for Biotechnology Information GenBank Sequence Read Archive (SRP130–364). Calculated with adegenet, the DAPC resulted in $K = 2$. Densities of individuals were plotted for varying discriminant functions, resulting in two clusters (Fig. 2A).

Estimates of K based on the cross-entropy criterion indicated that $K = 2$ had the highest likelihood in LEA (Fig. 2B). Both DAPC and the cross-entropy criterion analyses clustered individuals into the same two discrete populations. Two ancestral populations were consistently recovered, so $K = 2$ was used for all further analyses.

Admixture analysis yielded similar proportions of the two ancestral populations for the individuals collected from the Antarctic Peninsula and the Ross Sea and a distinctive difference in proportions from individuals collected from the Amundsen Sea. The Antarctic Peninsula and Ross Sea individuals were dominated by one ancestral population, and the Amundsen Sea individuals were dominated by the other ancestral population (Fig. A1).

Mantel tests were also executed to determine whether isolation by distance was occurring in this data set, but we did not find a significant relationship between genetic structure (represented by corrected F_{ST} values for samples from each
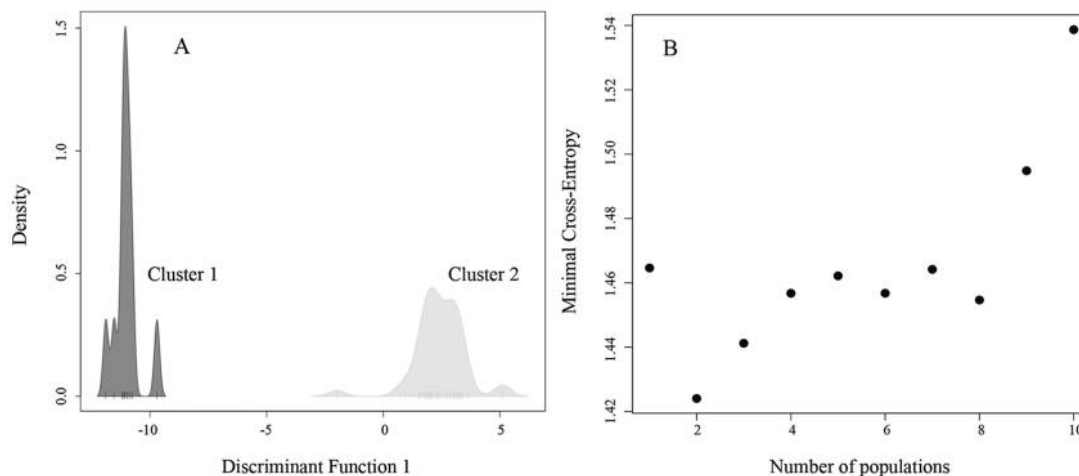

Figure 2. (A) Plot of single discriminant function created in discriminant analysis of principle components. Densities of individuals were plotted for varying discriminant functions. (B) Two populations were recovered with the Landscape and Ecological Associations 1.6.0 (LEA) package (Frichot and François, 2015).

geographic region, calculated with HIERFSTAT) and distance between each region (measured with the ruler tool in Google Earth Pro) (Fig. A2; Rousset, 1997; Spong and Creel, 2001). The regression of the ACC and the trans-Antarctic seaway measured by Mantel tests was not significant ($R^2 = 0.117$, $P = 0.506$ and $R^2 = 0.037$, $P = 0.713$, respectively; Fig. A2). Additional Mantel tests were conducted between collection sites within the east and west coasts of the Antarctic Peninsula, the Amundsen Sea, and the Ross Sea, and none were significant ($R^2 = 0.104$, $P = 0.532$; $R^2 = 0.437$, $P = 0.153$; $R^2 = 0.037$, $P = 0.589$; $R^2 = 0.052$, $P = 0.321$, respectively; Fig. A3).

The resulting data set and output file from *Stacks* revealed that only 2.5% of all SNPs had alignment stacks between the two "populations" of what we assumed was a single species based on morphology, knowledge of phenotypic plasticity of the species, and collection sites. The output file contained 3086 SNP loci, but only 78 loci had alignment stacks between the two "populations," with fixed alleles at 8 loci. When individuals from "population" 1 were analyzed, 2580 loci were coded for. When individuals from "population" 2 were analyzed with *Stacks*, 1095 loci were coded for. Furthermore, HIERFSTAT was used to calculate a Cavalli-Sforza and Edwards chord genetic distance between the two groups, which amounted to 0.334, demonstrating divergence greater than what would be expected within a single species (Frankham *et al.*, 2010; Krabbe *et al.*, 2010; Harder *et al.*, 2016). Only SNP alignments between populations (not between species) should be analyzed together. The two "populations" were first analyzed as one species with two populations, until we realized they were two different species, and then they were analyzed separately. Due to this divergence, the two "populations" were considered two putative species and analyzed as such from this point forward.

The two putative species were stratified geographically, with the 12 individuals of putative species 1 collected within the Amundsen Sea and the remaining 49 individuals of putative species 2 surrounding the Antarctic Peninsula and in the Ross Sea (Fig. 1). Each putative species was individually analyzed, as described above, in an attempt to identify population structures within each putative species. Adegenet and LEA identified both putative species to have $K = 1$ and $K = 1$ within each locality (Fig. A4). Admixture analyses and PCAs are not informative with $K = 1$ and thus were not conducted. Instead, F_{ST} statistics were calculated for each putative species and supported low population structuring within each group. The F_{ST} calculated among putative species 1 sample sites (collection latitude and longitude; Table A1) is 0.0591 ($P \leq 0.01$), and the F_{ST} calculated for putative species 2 sample sites is 0.0304 ($P = 0.027$). Pairwise F_{ST} values among sample sites in the Antarctic Peninsula and Ross Sea regions were lower, but with significant P-values (Table 1). As could be expected, pairwise F_{ST} values were higher and had significant P-values when either the Antarctic Peninsula

or the Ross Sea individuals were compared to the Amundsen Sea individuals (Table 1). A subsequent screening of these individuals found no distinct morphological characteristics separating the two groupings, identified all individuals as *N. australe*, and thus supported the notion of two cryptic species within the *N. australe* individuals included in this study.

Discussion

In this study, two geographically structured *Nymphon* species, thought initially to both be *Nymphon australe*, were recovered in the Western Antarctic. *Nymphon australe* Hodgson, 1902 has been previously described as a circumpolar species with presence/absence data (Munilla and Soler-Membrives, 2009); and more recently, molecular studies have found it to be a geographically structured circumpolar species, by using a coarser molecular data set (*COI*; Soler-Membrives *et al.*, 2017). Also using the coarser molecular data set (*COI* and *16S*), Mahon *et al.* (2008) supported the possibility of two previously unrecognized *Nymphon* species initially identified (morphologically) as *N. australe* in the Antarctic Peninsula region.

A possible explanation for undetected *N. australe* speciation is that most sea spiders collected from the Southern Ocean are identified as *N. australe*, even though it has long been recognized as a species with wide variability of morphology (Child and Cairns, 1995). Difficulty in species delineation within the Nymphonidae stems from variability of morphology and few physical traits useful to taxonomic analyses (Hedgpeth, 1947, 1955; Arnaud and Bamber, 1987; Arango, 2003). For example, a specimen presented as *Colossendeis* sp. was later found, with the sequenced mitochondrial genome of *Colossendeis megalonyx*, to be misidentified, and the identification was corrected to a nymphonid species (Dietz *et al.*, 2011). The two *Nymphon* species delineated with *COI* and *16S* by Mahon *et al.* (2008) identified both *Nymphon* species 1 and species 2 as *N. australe* when observed according to morphological traits alone.

Previous findings (Munilla and Soler-Membrives, 2009; Soler-Membrives *et al.*, 2017) of circumpolarity were not

Table 1

Pairwise fixation index (F_{ST}) values and P-values presented by locality and calculated with HIERFSTAT

F_{ST} and P-values	Amundsen Sea	Ross Sea	East Peninsula	West Peninsula
Amundsen Sea	. . .	0.132	0.116	0.112
Ross Sea	<0.001	. . .	0.031	0.030
East Peninsula	<0.001	0.007	. . .	0.007
West Peninsula	<0.001	0.040	0.646	. . .

F_{ST} values were calculated with Weir and Cockerham's estimate (1984) and are the values on the top right portion of the table. *P*-values are reported on the bottom left portion of the table.

supported by the genome-wide SNP data set produced in this study. However, this is not entirely unexpected because SNP data provide a much higher level of molecular resolution and genetic subdivision than had been previously noted. Importantly, the Soler-Membrives *et al.* (2017) study did not sample the Bellingshausen and Amundsen Seas, which is where our data found the first *N. australe* lineage. Inspection of mitochondrial markers has resulted in the discovery of cryptic or unrecognized species that overturns circumpolarity findings for many other Antarctic invertebrates, such as amphipods (Held, 2003; Held and Wägele, 2005; Havermans *et al.*, 2011), isopods (Raupach and Wägele, 2006), bivalves (Linse *et al.*, 2007), crinoids (Wilson *et al.*, 2007), and cephalopods (Allcock *et al.*, 2011). Possible cryptic speciation was also uncovered in other pycnogonid groups previously considered circumpolar (Krabbe *et al.*, 2010; Dömel *et al.*, 2017).

Geographic structuring of the postulated two different *N. australe* species is supported in Western Antarctic waters because sample locations coincide with the separation of individuals by SNP loci (Fig. 1). Genetic connectivity is also supported by F_{ST} values between individuals sampled from the Ross Sea and the Antarctic Peninsula, encompassing a distance of more than 5000 km (Table 1). The long-distance genetic connectivity excluding the Amundsen Sea can potentially be explained by the fact that on the western side of the continent, the ACC travels past the Ross Gyre and does not reach the continental shelf again until the peninsula (Tynan, 1998). Rafting on other organisms, such as a food source, provides sea spiders a potential way to travel long distances along currents (Fraser *et al.*, 2010, 2013; Leese *et al.*, 2010; Nikula *et al.*, 2010). This phylogeographic pattern was uncovered with RADseq techniques applied to a benthic brittle star population also sampled from Western Antarctica (Galaska *et al.*, 2017b).

An alternative hypothesis that could instead explain the phylogeographic patterns uncovered for *N. australe* included in this study, as well as other Southern Ocean species of brittle star (Galaska *et al.*, 2017b), bivalve (Linse *et al.*, 2007), and octopus (Allcock *et al.*, 2011), is a connection between the Ross and Weddell Seas. Geological data suggest that the West Antarctic ice sheet collapse and formation of trans-Antarctic seaways has occurred in the past and may occur in the future (Naish *et al.*, 2009; Pollard and DeConto, 2009). Trans-Antarctic passages connecting the Ross and Weddell Seas have existed in the recent past (125,000 years ago to 1.1 mya), facilitating organismal dispersal (Barnes and Hillenbrand, 2010). If dispersal occurred by means of a trans-Antarctic passage, putative species 2 would have dispersed over a distance of 3050 km to connect the individuals present in both the Antarctic Peninsula and the Ross Sea.

In this study, an overall homogenous population was not recovered for *N. australe*, indicating some restriction of genetic connectivity. However, similar to previous studies (Mahon *et al.*, 2008; Arango *et al.*, 2011; Soler-Membrives *et al.*, 2017), restriction to gene flow is not absolute, as putative species 2 may exhibit an extensive dispersal. *Nymphon australe* has a brooding, lecithotrophic larval protonymphon stage that limits dispersal and is not eurybathic. Thus, we are uncertain whether long-distance genetic connectivity is due to glacial activity, the ACC, other currents, the ability to raft on other organisms, or other unknown methods of mobility (Moon *et al.*, 2017). We are also uncertain whether putative species 2 may be exhibiting a phylogenetic pattern representative of a past connection of the Ross and Weddell Seas. Additionally, the influence of genetic connectivity through introgression or hybridization and selective effects on these species cannot be ruled out.

Discovery of cryptic species is paramount to the true understanding of species distributions. Such understanding contributes to comprehension of the planet's past changes in climate and geology. Knowledge of how past conditions impacted gene flow can contribute to predictions for future reactions to major environmental fluctuations in a rapidly changing environment, such as in polar regions afflicted by polar warming amplification (Taylor *et al.*, 2013). This study demonstrates the ability of RAD-based SNP markers to detect geographically stratified, cryptic species missed by other lower-resolution markers. Also, this work points to the importance of geographic sampling and the continuing need to sample the full range of organisms with high-resolution markers.

Data Accessibility

Raw reads for 2b restriction site-associated DNA single-nucleotide polymorphism data are deposited in the National Center for Biotechnology Information GenBank Sequence Read Archive (SRP130–364).

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Appendix

Table A1

Specimens collected, with localities, latitudes, longitudes, depths, and sample identifications

Species	Locality	Latitude	Longitude	Depth (m)	Sample ID
-	Bellingshausen	70°48′42.81″S	92°31′18.24″W	430	565
-	Bellingshausen	70°48′42.81″S	92°31′18.24″W	430	566
Putative species 1	Amundsen	72°28′95.08″S	104°33′77.10″W	591	5
Putative species 1	Amundsen	72°28′95.08″S	104°33′77.10″W	591	7
Putative species 1	Amundsen	72°46′83″S	104°33′23″W	496	13
-	Amundsen	72°46′83″S	104°33′23″W	496	17
-	Amundsen	72°46′83″S	104°33′23″W	496	18
-	Amundsen	72°46′83″S	104°33′23″W	496	19
Putative species 1	Amundsen	73°43′29.16″S	103°37′01.16″W	699	34
Putative species 1	Amundsen	73°43′29.16″S	103°37′01.16″W	699	35
Putative species 1	Amundsen	73°43′29.16″S	103°37′01.16″W	699	36
-	Amundsen	73°43′29.16″S	103°37′01.16″W	699	37
Putative species 1	Amundsen	73°43′29.16″S	103°37′01.16″W	699	52
-	Amundsen	72°12′15.15″S	103°35′46.81″W	341	55
-	Amundsen	72°12′15.15″S	103°35′46.81″W	341	56
-	Amundsen	72°12′15.15″S	103°35′46.81″W	341	57
-	Amundsen	72°12′15.15″S	103°35′46.81″W	341	58
-	Amundsen	72°12′15.15″S	103°35′46.81″W	341	59
Putative species 1	Amundsen	73°9′31.90″S	129°53′41.85″W	516	66
Putative species 1	Amundsen	73°9′31.90″S	129°53′41.85″W	516	69

Table A1 (*Continued*)

Species	Locality	Latitude	Longitude	Depth (m)	Sample ID
Putative species 1	Amundsen	73°17′47.98″S	129°11′32.80″W	655	90
Putative species 1	Amundsen	73°17′47.98″S	129°11′32.80″W	655	92
Putative species 1	Amundsen	73°17′47.98″S	129°11′32.80″W	655	97
-	Amundsen	73°9′31.90″S	129°53′41.85″W	516	589
-	Amundsen	73°9′31.90″S	129°53′41.85″W	516	591
Putative species 2	Ross	76°20′28.38″S	170°51′1.78″W	764	205
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	272
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	273
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	275
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	276
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	277
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	278
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	279
Putative species 2	Ross	75°50′0.47″S	166°30′19.78″E	552	332
Putative species 2	Ross	75°50′0.47″S	166°30′19.78″E	552	333
Putative species 2	Ross	75°50′0.47″S	166°30′19.78″E	552	334
-	Ross	75°50′0.47″S	166°30′19.78″E	552	335
-	Ross	75°50′0.47″S	166°30′19.78″E	552	336
Putative species 2	Ross	76°54′13.68″S	169°57′54.90″E	764	638
Putative species 2	Ross	76°54′13.68″S	169°57′54.90″E	764	639
Putative species 2	Ross	75°50′0.47″S	166°30′19.78″E	552	650
Putative species 2	Ross	75°50′0.47″S	166°30′19.78″E	552	651
Putative species 2	Ross	75°50′0.47″S	166°30′19.78″E	552	652
Putative species 2	Ross	75°50′0.47″S	166°30′19.78″E	552	654
Putative species 2	Ross	74°41′0.14″S	168°28′0.19″E	513	656
Putative species 2	Ross	74°10′55.12″S	166°39′39.70″E	390	657
Putative species 2	Ross	74°10′55.12″S	166°39′39.70″E	390	658
Putative species 2	Ross	78°3′47.66″S	169°59′28.14″W	549	625
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	631
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	632
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	633
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	634
Putative species 2	Ross	76°14′42.94″S	174°30′14.83″E	604	635
Putative species 2	East Peninsula	64°2′6.60″S	56°43′41.70″W	290	427
Putative species 2	East Peninsula	64°2′6.60″S	56°43′41.70″W	290	428
Putative species 2	East Peninsula	64°2′6.60″S	56°43′41.70″W	290	429
Putative species 2	East Peninsula	64°2′6.60″S	56°43′41.70″W	290	430
-	East Peninsula	64°2′6.60″S	56°43′41.70″W	290	431
Putative species 2	East Peninsula	64°2′6.60″S	56°43′41.70″W	290	432
Putative species 2	East Peninsula	64°2′6.60″S	56°43′41.70″W	290	435
Putative species 2	East Peninsula	64°08.357′S	56°51.994′W	-	451
Putative species 2	East Peninsula	63°41′8.82″S	56°51′32.40″W	400	464
Putative species 2	East Peninsula	63°41′8.82″S	56°51′32.40″W	400	465
-	East Peninsula	63°41′8.82″S	56°51′32.40″W	400	466
Putative species 2	East Peninsula	63°41′8.82″S	56°51′32.40″W	400	467
Putative species 2	East Peninsula	63°45′13.38″S	55°41′2.34″W	334	484
Putative species 2	East Peninsula	63°45′13.38″S	55°41′2.34″W	334	492
Putative species 2	East Peninsula	63°45′13.38″S	55°41′2.34″W	334	495
Putative species 2	East Peninsula	63°45′13.38″S	55°41′2.34″W	334	496
-	West Peninsula	64°24′40.32″S	61°57′47.40″W	664	402
-	West Peninsula	64°24′40.32″S	61°57′47.40″W	664	403
-	West Peninsula	64°24′40.32″S	61°57′47.40″W	664	404
-	West Peninsula	64°24′40.32″S	61°57′47.40″W	664	405
-	West Peninsula	64°24′40.32″S	61°57′47.40″W	664	412
-	West Peninsula	64°24′40.32″S	61°57′47.40″W	664	413
-	West Peninsula	64°24′40.32″S	61°57′47.40″W	664	414
-	West Peninsula	64°24′40.32″S	61°57′47.40″W	664	415
-	West Peninsula	63°48′19.95″S	60°28′44.70″W	428	422
Putative species 2	West Peninsula	63°48′19.95″S	60°28′44.70″W	428	423
-	West Peninsula	63°48′19.95″S	60°28′44.70″W	428	424

Table A1 (*Continued*)

Species	Locality	Latitude	Longitude	Depth (m)	Sample ID
-	West Peninsula	63°48′19.95″S	60°28′44.70″W	428	426
Putative species 2	West Peninsula	65°1′15.12″S	64°25′30.12″W	312	546
Putative species 2	West Peninsula	64°38′24.00″S	64°14′43.38″W	312	547
Putative species 2	West Peninsula	64°38′24.00″S	64°14′43.38″W	312	548
Putative species 2	West Peninsula	64°38′24.00″S	64°14′43.38″W	312	549
-	West Peninsula	64°38′24.00″S	64°14′43.38″W	312	550
-	West Peninsula	64°38′24.00″S	64°14′43.38″W	312	551
Putative species 2	West Peninsula	64°38′24.00″S	64°14′43.38″W	312	556
-	West Peninsula	64°38′24.00″S	64°14′43.38″W	312	559
Putative species 2	West Peninsula	64°38′24.00″S	64°14′43.38″W	312	560
Putative species 2	West Peninsula	64°38′24.00″S	64°14′43.38″W	312	561
Putative species 2	West Peninsula	65°5′12.90″S	65°48′31.98″W	202	665

Species are labeled according to designation in adegenet (Jombart, 2008; Jombart and Ahmed, 2011) and Landscape and Ecological Associations 1.6.0 (LEA) package (Frichot and François, 2015) analyses or with a dash if filtered out before assignment to a particular species.
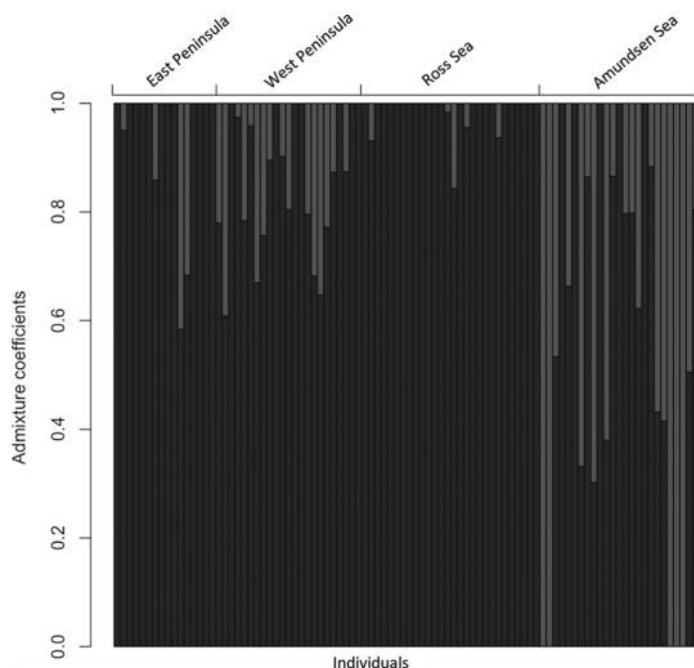

Figure A1. Admixture plotted in the Landscape and Ecological Associations 1.6.0 (LEA) package (Frichot and François, 2015), with admixture coefficients on the *Y*-axis and every individual along the *X*-axis separated by regions labeled at the top. Discriminant analysis of principle components and LEA analyses delineate the same individuals into the same populations.
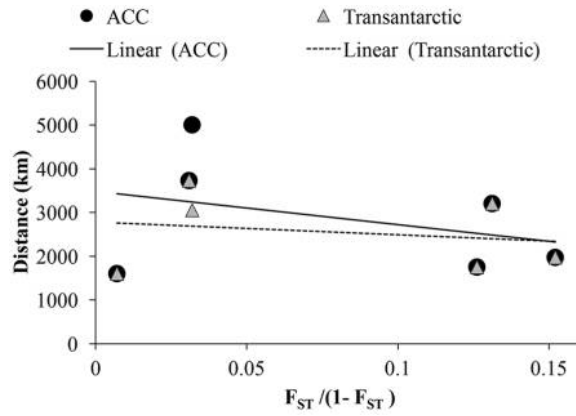
Figure A2. Mantel test with corrected fixation index (F_{ST}) values and distances (km) between the Amundsen Sea, the Ross Sea, and the east and west coasts of the Antarctic Peninsula. The Antarctic Circumpolar Current (ACC) measure represents a distance measured between the east coast of the Antarctic Peninsula and the Ross Sea by going around the continent as the ACC travels. The trans-Antarctic measure represents a distance measured over land between the east coast of the Antarctic Peninsula and the Ross Sea.
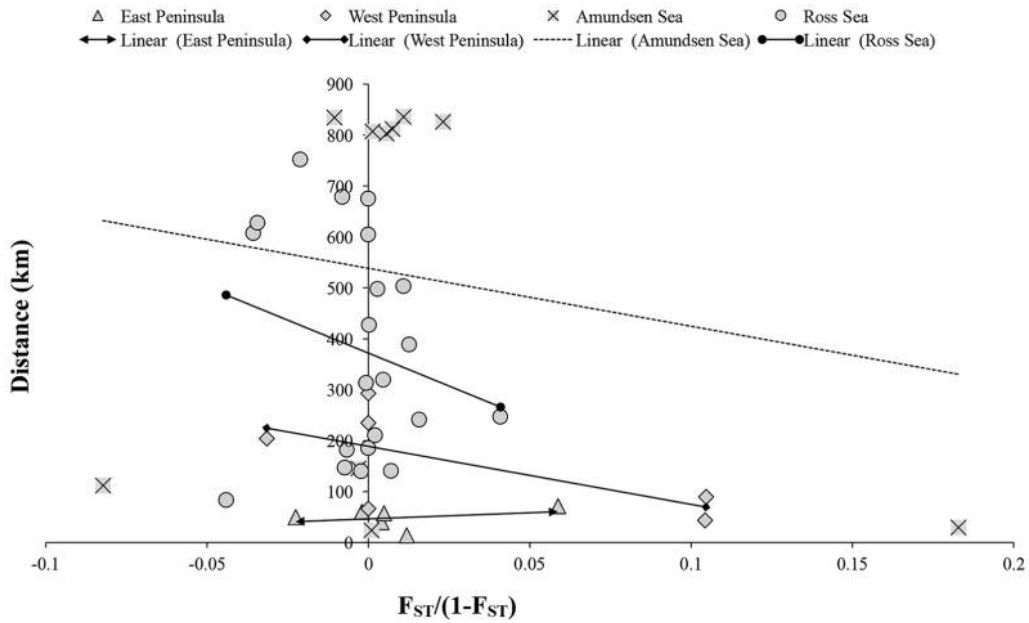

Figure A3. Mantel test with corrected fixation index (F_{ST}) values and distances (km) between sites within the Amundsen Sea, the Ross Sea, and the east and west coasts of the Antarctic Peninsula.
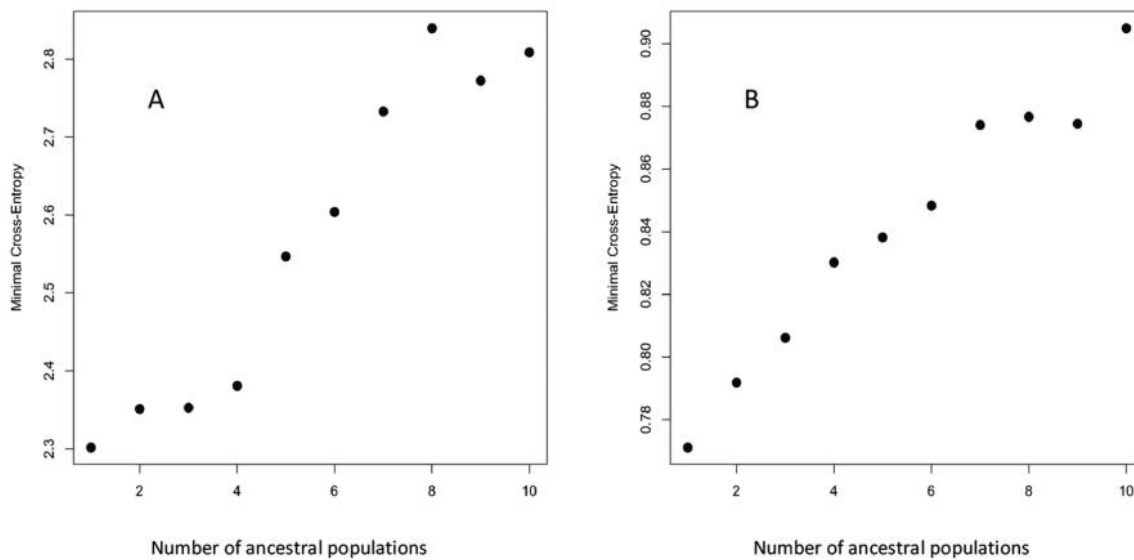
Figure A4. (A) One population ($K = 1$) was recovered with the Landscape and Ecological Associations 1.6.0 (LEA) package (Frichot and François, 2015) for the first putative species. (B) One population ($K = 1$) was recovered with LEA for the second putative species.