

Against the flow: evidence of multiple recent invasions of warmer continental shelf waters by a Southern Ocean brittle star

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The Southern Ocean is anomalously rich in benthos. This biodiversity is native, mostly endemic and perceived to be uniquely threatened from climate- and anthropogenically-mediated invasions. Major international scientific effort throughout the last decade has revealed more connectivity than expected between fauna north and south of the world's strongest marine barrier—the Polar Front (the strongest jet of the Antarctic Circumpolar Current). To date though, no research has demonstrated any radiations of marine taxa out from the Southern Ocean, except at abyssal depths (where conditions differ much less). Our phylogeographic investigation including mitochondrial sequences of 135 specimens of one of the most ubiquitous and abundant clades at high southern latitudes, the ophiuroids (brittlestars), shows that one of them, *Ophiura lymani*, has gone against the flow. Remarkably our phylogenetic and coalescent analyses suggest that *O. lymani* has successfully invaded the South American shelf from Antarctica at least three times, in recent (Pleistocene) radiation. Many previous studies have demonstrated links within clades across the Polar Front this is the first in which northwards directional movement of a shelf-restricted species is the only convincing explanation. Rapid, recent, regional warming is likely to facilitate multiple range shift invasions into the Southern Ocean, whereas movement of cold adapted fauna (considered highly stenothermal) out of the Antarctic to warmer shelves has, until now, seemed highly unlikely.

Keywords: adaptation, climate change, cryptic species, Ophiuroidea, range expansion, radiation, phylogeography, Pleistocene

Introduction

Marine animals inhabiting continental shelves can disperse widely over ecological time scales due to extensive shelf connectivity, current regimes, and reasonably consistent ocean chemistry (Paulay and Meyer, 2006). Nevertheless, some local endemism occurs in most continental shelf regions (Sanford and Kelly, 2011). Temperature is a strong predictor of biogeographic regions (Belanger et al., 2012) and studies demonstrate that given a warming climate many marine shelf species track pole-wards and into deeper, cooler habitats (Norberg et al., 2012; Pinsky et al., 2013; Hiddink et al., 2015). Shelf areas within the Southern Ocean are unusually isolated compared with those north of

the Southern Ocean. They are surrounded by deep seas (>2000 m depth), the strongest oceanic current on the planet, the Antarctic Circumpolar Current (ACC) (Whitworth, 1980; Orsi et al., 1995), and encircled by the Polar Front, a narrow jet within the ACC, across which there are steep gradients in temperature and salinity. These isolating conditions, established in the Oligocene (Barker and Thomas, 2004), have left a signature of high endemism of the Southern Ocean shelf fauna (Dell, 1972; Clarke and Crame, 1989; Griffiths et al., 2009).

Over the ~24 million years since the opening of the Drake Passage, the Miocene Antarctic benthos has had to respond to the cooling conditions by either adapting *in situ* or range shifts into appropriate environmental conditions. This for instance could have resulted in species ranges being restricted to the northern continental shelves of South Georgia and South America, while Antarctic shelf populations went extinct (Clarke and Crame, 1989). The onset of glaciation caused extinction in most, but not all, of the terrestrial flora and fauna (Convey et al., 2008), but in the more constant marine environment, much of the fauna has adapted successfully and has become comparably rich (Clarke and Johnston, 2003). There has long been debate regarding the origins of the Antarctic fauna and how much movement there has been of fauna into and out of the Antarctic realm over time since its isolation (Hedgpeth, 1969; Dell, 1972; Clarke and Crame, 1989; Clarke et al., 2005; Barnes et al., 2006; Griffiths et al., 2009). Although there is clearly biogeographic evidence of a South American influence in the Southern Ocean fauna (Griffiths et al., 2009), phylogenetic studies suggest that octopus (Strugnell et al., 2008), and pleurobranch gastropods (Göbbeler and Klussmann-Kolb, 2010) have radiated out of the Antarctic, possibly assisted by the initiation of the formation of Antarctic bottom water, around the time of the Oligocene cooling. There is also molecular evidence suggesting that some Antarctic species and their northern lineages diverged much more recently in the Pliocene or Pliocene (Page and Linse, 2002; Hunter and Halanych, 2008; González-Wevar et al., 2010; Krabbe et al., 2010; Poulin et al., 2014), while several species are currently thought to be distributed on either side of the Polar Front indicating recurrent gene flow (see Sands et al., 2013 Supplementary Material for ophiuroid examples).

The recently observed increase in Southern Ocean surface temperatures (Meredith and King, 2005; Whitehouse et al., 2008; Turner et al., 2014) is suggested to have serious implications for Southern Ocean biota. The fauna is widely considered to be cold adapted with no option of migration except to the deep sea (Peck, 2005), which in itself requires specific adaptations for survival (Brown and Thatje, 2015). Warming of the Southern Ocean is also likely to facilitate invasion of species from more northern regions. This is most likely to be observed first on the more northern shelf regions of the Southern Ocean, especially the large micro-continental shelf regions of South Georgia and Shag Rocks (Barnes et al., 2009; Hogg et al., 2011; Morley et al., 2014) and the even larger but less studied waters of the Kerguelen Plateau. Despite being located south of the Polar Front, South Georgia is eastwards of the tip of South America and directly affected by the ACC flowing across from the South American Shelf. The benthic fauna of the South Georgia shelf has been described as “Antarctic”

(Hogg et al., 2011), although biogeographic work indicates that there is substantial South American influence (Griffiths et al., 2009). There are several shelf-restricted brittle star (Ophiuroidea) species that are located on both the Patagonian and South Georgian shelves (Sands et al., 2013). Given the strong ACC with the potential to promote gene flow we expect a pattern consistent with the South American influence hypothesis that predicts (1) decreasing genetic diversity from the Patagonian Shelf to Antarctic Shelf; (2) older lineages i.e., more basal clades in a phylogeny, will consist of Patagonian Shelf specimens and (3) tests for gene flow reveal asymmetrical migration in the direction of the ACC from the Patagonian shelf population to South Georgia. Using a fragment of the mitochondrial cytochrome c oxidase subunit 1 gene (CO1) amplified from collections of the brittle star *Ophiura lymani* and its sister species *Ophiura carinifera*, acquired throughout their ranges around the Southern Ocean, we tested these expectations under the hypothesis of South American origin against the alternative hypothesis of Antarctic origin.

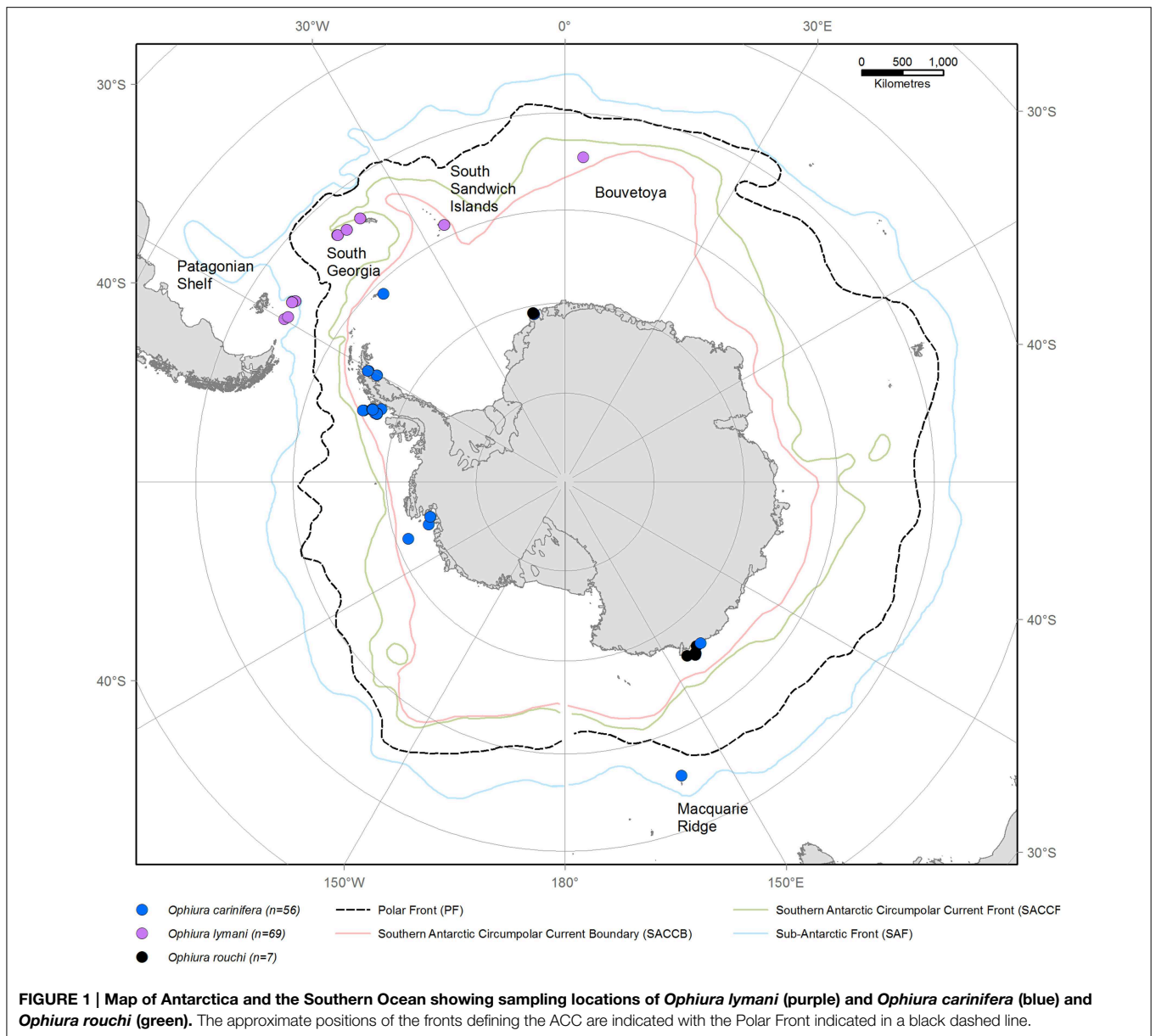
Materials and Methods

Our approach was to first test for genetic structure within our samples using phylogenetic methods. We then conducted tests using coalescent techniques to best identify the processes that underlying the phylogenetic patterns identified.

Specimens of *O. lymani* and *O. carinifera* were collected on several international expeditions (JR 144, JR179, JR230, PS77, ANDEEP, ICEFISH, CEAMARC, and MacRidge 2) using Agassiz Trawls. Sampling locations are illustrated in **Figure 1**. Samples were sorted immediately on collection and stored in 96% ethanol. Individuals were identified morphologically to species level.

Tissue samples taken from arm tips were used for DNA extraction. DNA extraction and sequencing of partial cytochrome c oxidase subunit 1 (CO1) was carried out by the Canadian Center for DNA Barcoding using the primers LCOech1aF1 and HCO2198 (Folmer et al., 1994), with sequences and metadata stored and made publically available on the Barcode of Life Data Systems (Ratnasingham and Hebert, 2007). Forward and reverse trace files were downloaded from the database and assembled and edited in CodonCode Aligner V5.0.1. Sequences were translated to ensure an open reading frame, and aligned using the MUSCLE (Edgar, 2004) alignment process. We included sequences from *Ophiura rouchi* as the most reasonable outgroup and to stabilize more basal nodes (Felsenstein, 1981) we included two further outgroups, *Ophiocten dubium* and *Ophioperla koehleri*.

Descriptive statistics produced using DnaSP v5.10.01 (Librado and Rozas, 2009) and Arlequin v3.5 (Excoffier et al., 2005). Departures from drift mutation equilibrium were tested by comparing estimates of nucleotide diversity with segregating sites—Tajima’s D (Tajima, 1989)—and by assessing observed vs. expected haplotype diversity—Fu’s F_s (Fu, 1996). Both these statistics are affected by population size changes as well as selection. Significance was assessed from 1000 coalescent simulations in DnaSP. We conducted mismatch analysis (Rogers and Harpending, 1992) to test whether the frequency



distribution of pairwise differences conformed with the random (ragged) distribution expected under equilibrium or whether the distribution appeared non-random as expected under past population change. *P*-values were generated by conducting 1000 bootstrap pseudoreplicates in Arlequin. Within and among clade distances were calculated in MEGA v5.2.2 (Tamura et al., 2011). Base compositional heterogeneity was examined using a match paired test in SeqVis v1.5 (Ho et al., 2006).

To visualize the change of effective size through time in each population, Bayesian Skyline (Drummond et al., 2005) and Bayesian Skyride plots (Minin et al., 2008) were produced in BEAST v1.8.1 (Drummond and Rambaut, 2007) using coalescent Skyline and Gaussian Markov random field Bayesian Sky Ride tree priors respectively, and a mutation rate of 2.48×10^{-8} (see below). Ten million generations of MCMC produced

10,000 trees. TRACER v1.6 (Rambaut et al., 2014) was used to check diagnostics of convergence and effective sample sizes of parameters, as well as to analyse the trees and produce the plots.

To determine the relationships among *O. lymani* populations and the closely related High Antarctic species *O. carinifera*, we produced a Maximum Likelihood estimate of phylogeny using RAxML v8.0.26 (Stamatakis, 2014) and a Bayesian estimate using the program MrBayes v3.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). In order to account for violations of assumptions of stationarity and heterogeneity we used JModeltest v2 (Darriba et al., 2012) and used AIC to determine that GTR+I+G was the most suitable substitution model. As proportion of invariant sites (I) is closely correlated with the gamma distribution (+G) we used the GTR+G option in RAxML, with 10 replicate runs on both a codon partitioned

dataset and a non-partitioned dataset. We ran 1000 “thorough” (as opposed to the “rapid” option) bootstrap pseudoreplicates to estimate node support. A codon partitioned dataset was used in our Bayesian analysis to better account for variation in substitution rates across sites and lineages. Each partition was set to estimate all parameters ($nst = 6$) effectively allowing a GTR model with a gamma distribution. Phylogenies were estimated using four replicates of four heated chains over 10^8 generations with a 25% burnin that ensured trees were sampled only once split frequencies fell below 0.01. Convergence and effective sample size estimates of parameters were checked using TRACER v1.6. An alternative visualization of the relationships among haplotypes of *O. lymani* sampled from the Patagonian shelf and the South Georgia/Shag Rocks shelf areas was generated in the form of a statistical parsimony network (Templeton et al., 1992) using the package TCS v1.21 (Clement et al., 2000). From this reduced dataset we constructed a Bayesian phylogeny as described above to be used as the gene tree estimate in coalescent simulations.

Species delimitation was explored using popular analytic techniques. Automatic barcode gap discovery (Puillandre et al., 2012) is a distance-based technique that explores a predicted “gap” in the frequencies of pairwise distances indicating the discrete change in intra- and interspecific evolutionary processes. Parameters P_{\min} (lowest expected distance to include a barcode gap), P_{\max} (maximum expected distance to include a barcode gap), X (relative width of the barcode gap), and distance correction (JC69, K2P, SimpleP) were adjusted to best encompass the range of variation in the data ($P_{\min} = 0.001$, $P_{\max} = 0.1$, $X = 0.1$, K2P distance). The resulting number of species groups was estimated after the stabilization of recursive partitioning of the data. The general mixed Yule coalescent (GMYC) method is a phylogenetic technique (Pons et al., 2006) that was implemented in the R package SPLITS (Fujisawa and Barraclough, 2013) using an ultrametric tree produced in the software BEAST v1.8.1 (Drummond and Rambaut, 2007) as described below.

Where results of non-monophyly between regions were observed we used a test of the cost of deep coalescence (cDC) events required to explain the data compared with that of distribution of coalescent trees simulated from the original gene tree. As the coalescent process is stochastic, any pattern inferred by phylogeny may be chance—rather than process—driven. In the software package Mesquite v3.01 (Maddison and Maddison, 2014) we constructed a species tree specifying a topology representing two populations: Patagonian shelf and South Georgian shelf. The Bayesian gene tree estimated from the reduced dataset was forced into the species tree and the cDC recorded. The same gene tree was used as a starting point to simulate 10,000 coalescent trees, each one forced to fit the species tree and the number of deep coalescent events noted. The cDC from the gene tree was compared to a distribution of the cDC from the simulations.

The likelihood of the exchange of migrants between the Patagonian shelf and South Georgia shelf was tested in Migrate v3.6.4 (Beerli, 2009) by examining the posterior distribution of migration estimates from an unrestricted

model allowing full symmetrical migration, and then by ranking competing migration models using thermodynamic integration with a Bayes factor test (Beerli and Palczewski, 2010). The models examined were symmetrical migration (PS<->SG), asymmetrical migration (PS->SG), (PS<-SG), panmixia (PS = SG) and no migration (PS-x-SG). Prior to running these models, Migrate-n settings were optimized using a full model (symmetrical migration) to ensure priors were appropriate and runs would converge. Final run conditions were 10 replicate long chains each with four heated chains with temperatures 1, 1.5, 3, 10^6 . Each chain was run for 10^6 generations, sampling every 100 generations.

The timing of divergence events was estimated using a strict molecular clock in BEAST v1.8.1 (Drummond and Rambaut, 2007). We allowed a GTR + G model on partitioned codon sites and a Yule process speciation prior. A lognormal prior was used for “clock rate” such that the median reflected the mutation rate per lineage per year (2.48×10^{-8}) as determined by Naughton et al. (2014) and 95% distribution between 1.2×10^{-8} and 8.7×10^{-8} thus covering a broad range of uncertainty. Sixteen test runs of 10^8 generations were used to test and adjust priors and their effects on convergence, mixing and parameter estimates.

All data and meta-data used in this study has been made publically available on BOLD Systems (www.boldsystems.org) under the project code DS-MS001 (DOI: [dx.doi.org/10.5883/DS-MS001](https://doi.org/10.5883/DS-MS001)) and on GenBank with accession numbers KR861569-KR86164.

Results

The Samples

A total of 69 *O. lymani* specimens were collected; 33 from the Patagonian shelf, 31 from the South Georgia/Shag Rocks shelf, three from South Sandwich Islands and two close to the remote Bouvetoya. Morphological examination of all specimens supported a single morpho-species.

O. carinifera, the sister taxon to *O. lymani*, is clearly identifiable by strongly elevated and granulated disc plates and radial shields (flattened without granules in *O. lymani*), and narrow dorsal arm plates with a serrated keel (wide without serrated rest in *O. lymani*). We included 56 specimens sampled from around the Antarctic continental shelf—from the Amundsen Sea, Bellingshausen Sea, Larsen shelf, South Orkney shelf and the south-east region of the Weddell Sea, east Antarctica near the Mertz Glacier and a specimen from the Macquarie Ridge. The Macquarie Ridge specimen (OPHAN310) was morphologically distinct from all others having a more flattened disc, similar to *O. lymani* and genetically divergent from all other *O. carinifera*, appearing to be the sole representative of a sister clade (Figure 2).

Molecular Diversity

Our alignment of 132 sequences consisted of 658 bases, of which 138 were variable and 97 were parsimony informative. As expected with mitochondrial sequence there was a bias toward Adenosine and Thymine residues (GC = 39.2%).

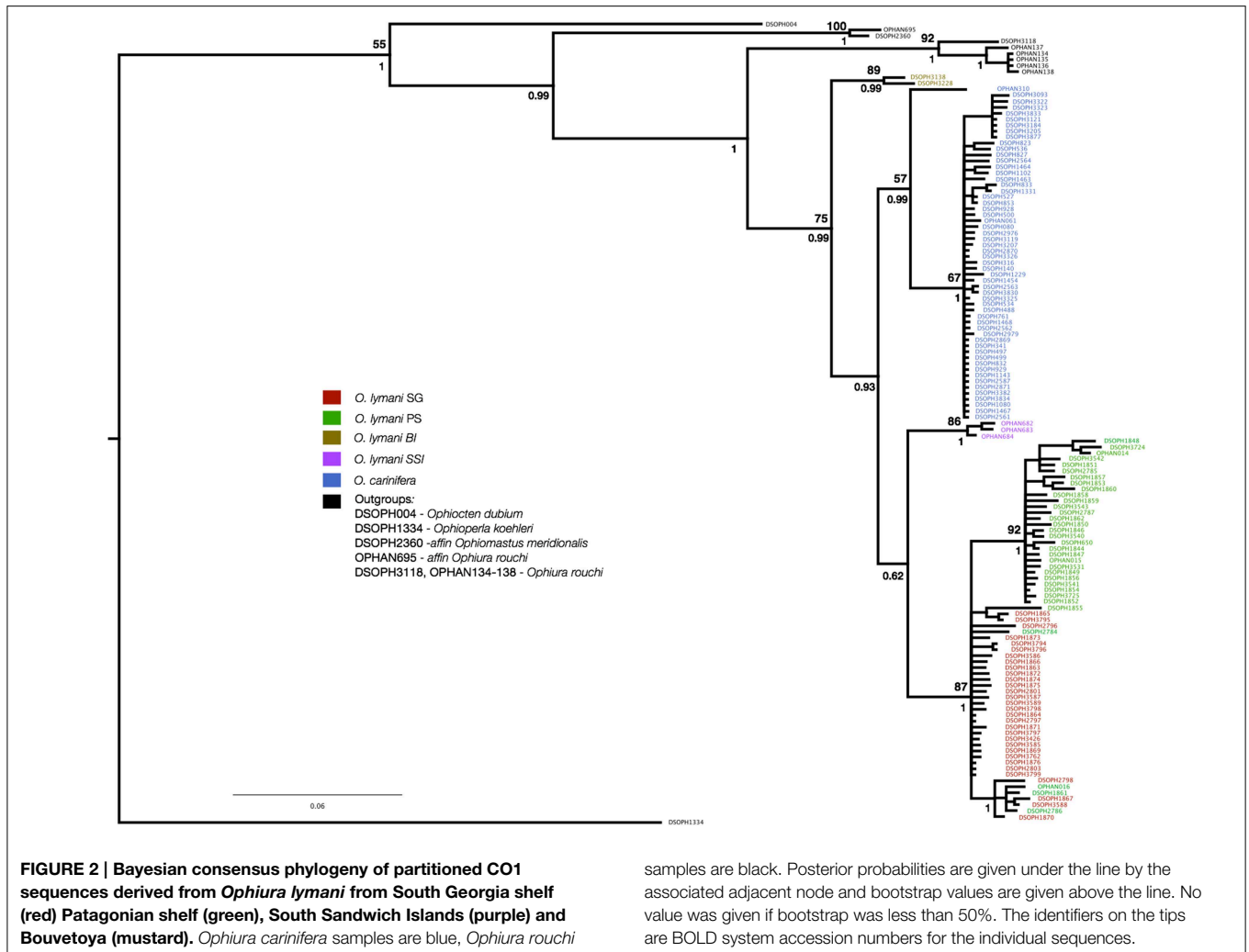


TABLE 1 | Summary statistics of molecular diversity.

Population	<i>n</i>	Prob	No. haplotypes	H_D	<i>S</i>	π	D_T	F_s	R_2	Max. K
<i>O. lymani</i> PS	33	0.9412	33	1	67	0.018	-1.09	-22.17**	0.073169	30
<i>O. lymani</i> SG	31	0.9375	27	0.989	43	0.010	-1.39	-15.62**	0.0637*	18
<i>O. carinifera</i> AS	55	0.9643	31	0.926	36	0.006	-1.86*	-5.05	0.0423**	12
<i>O. carinifera</i> MR	1	0	1	na	na	na	na	na	na	na
<i>O. lymani</i> SSI	3	0.5	3	1	5	0.005	na	na	na	4
<i>O. lymani</i> BI	2	0.325	2	1	8	0.012	na	na	na	8

* $P > 0.05$, ** $P > 0.01$.

n, Number of individuals; Prob, Probability of having captured the deepest coalescent event; No. haplotypes, Number of haplotypes; H_D , Haplotype diversity; *S*, Number of segregating sites; π , Nucleotide diversity; D_T , Tajima's *D*; F_s , Fu's F_s statistic; R_2 , RamosOnsins and Rozas' R_2 statistic; Max K, Maximum number of nucleotide differences between any two sequences within the population.

Haplotype diversity (H_D) was exceptionally high in all populations of *O. lymani* and *O. carinifera* (Table 1). Tajima's *D* was negative, although not significantly different from zero in *O. lymani*. Significant negative values as seen in *O. carinifera* may indicate either purifying selection or population size change. Fu's F_s was also negative as to be expected given the high haplotype diversity. Fu's F_s was highly significant in the

O. lymani populations from the Patagonian shelf and South Georgian shelf, both of which have very high haplotype diversity. Significant R_2 -values also suggest population expansion, but only in *O. carinifera* and the South Georgia population of *O. lymani*. The frequency distribution of pairwise differences under a constant population is expected to be largely random, whereas the expectation under population growth is for a smooth

curve. Although non-significant, the two *O. lymani* populations, particularly the South Georgia population, appeared to more closely fit the expectations of population expansion (Figure 3). Coalescent-based demographic reconstruction using Bayesian Skyline and Skyride plots indicated a population increase over the past few glacial cycles in all three populations (Figure 4). The Skyline plots show a sharp increase in population size starting 100+ ka, whereas smoothing algorithms in the Skyride plots indicate a steady increase over time.

Phylogenetic Reconstruction

Phylogenetic reconstruction identified strongly supported clades of geographically defined populations of *O. lymani*, with the exception of five individuals sampled from the Patagonian shelf that grouped with the South Georgian shelf individuals, and a single Antarctic-wide clade including two lineages of *O. carinifera* (Figure 2). The low support of the node containing the South Sandwich Islands, South Georgia and Patagonian Shelf clades (pp. 0.62) persisted despite trialing various plausible evolutionary models and partitioning schemes. Low support for nodes should not always be interpreted as phylogenetic failure, but may rather reflect a genuine (hard) polytomy due to a radiation or series of speciation events between which there was insufficient time to accumulate mutational variation to permit accurate reconstruction (Hoelzer and Meinick, 1994).

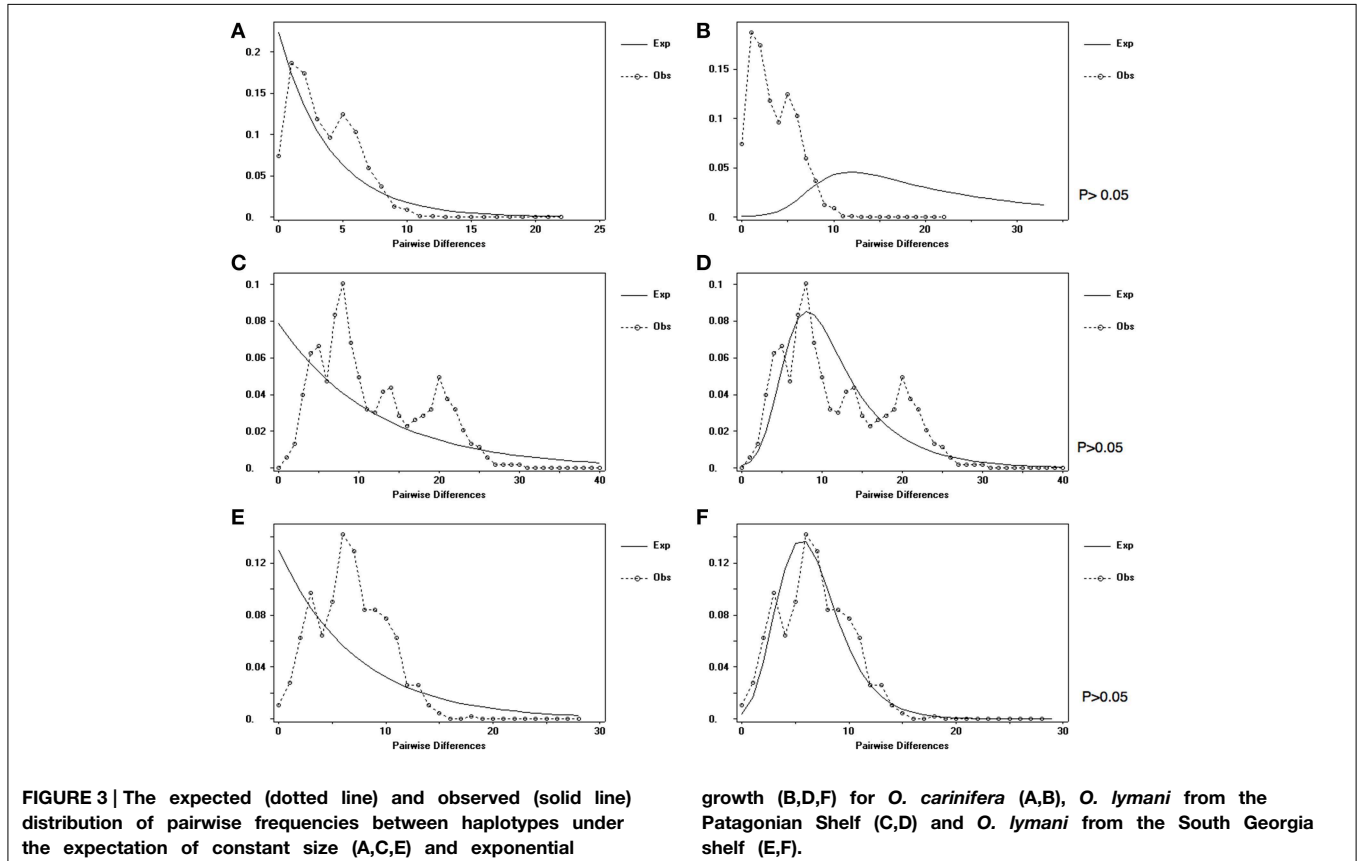
The statistical parsimony network highlighted five individuals sampled from the Patagonian shelf that either originated from the

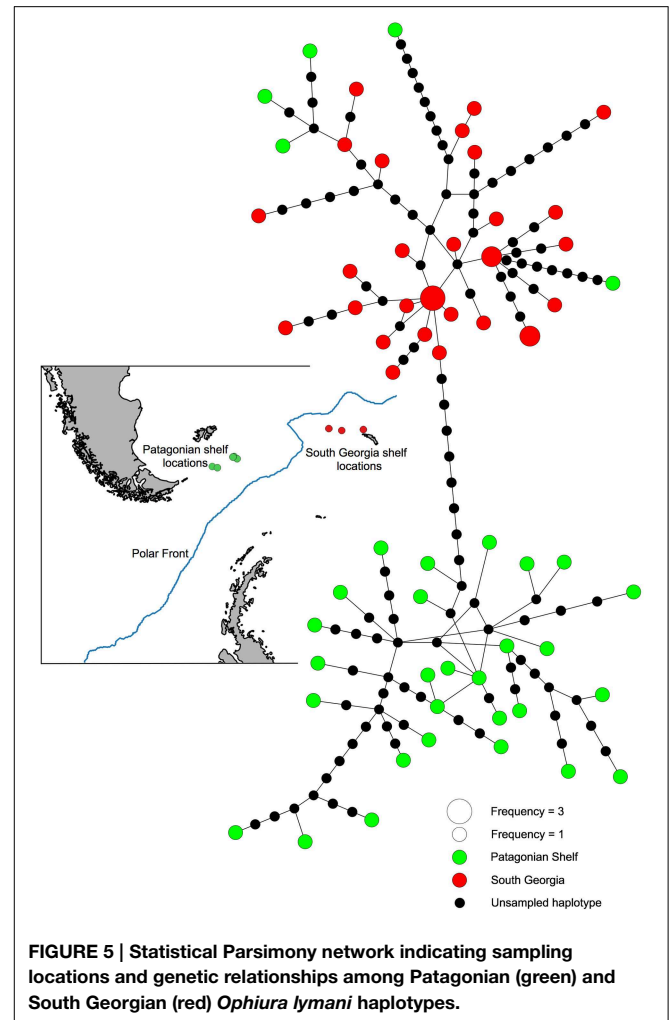
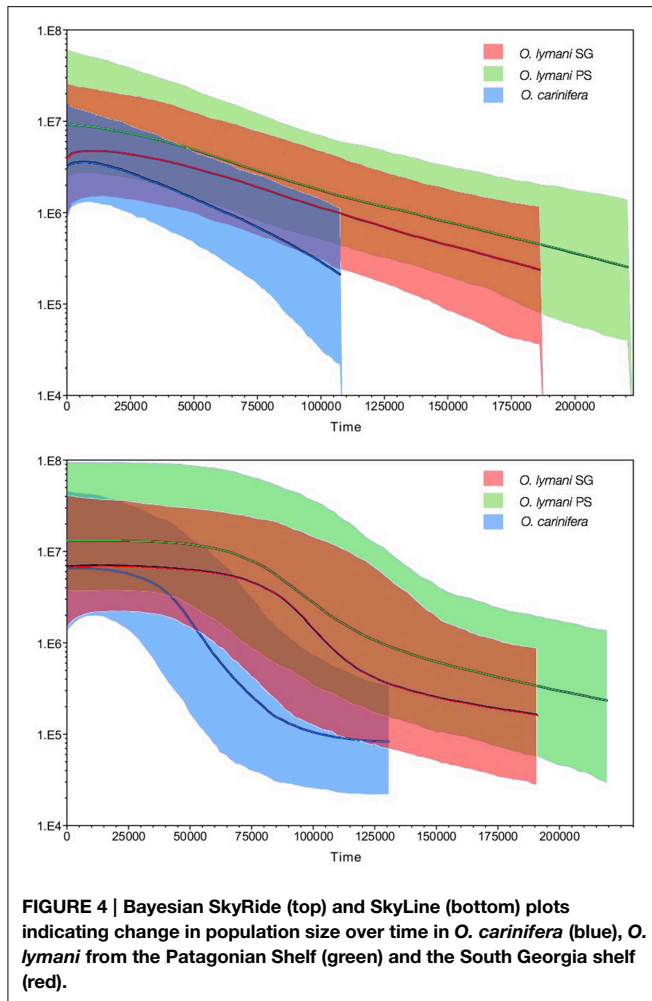
South Georgia clade, or are retained ancient lineages indicative of incomplete lineage sorting (Figure 5). In order to determine the likelihood of these lineages being retained, we determined the cost of deep coalescence (cDC) required to explain the tree. The distribution of cDC of the simulated trees had a mean of 48 (41–55, 95% CI). The cDC estimated from the Bayesian phylogeny was 4, well outside of this distribution, indicating that chance alone is very unlikely to explain the low number of deep coalescences required to produce this species tree from the gene tree.

Species Delimitation

Genetic distances between populations were considerably greater than within-population distances, and generally greater than are expected for conspecific populations (Table 2). Visual inspection suggests that clades representing *O. lymani* from Bouvet, the South Sandwich Islands and the Patagonian shelf/South Georgia are likely to be three distinct species, considering that the clearly morphologically distinct *O. carinifera* is grouped within the pan *O. lymani* clade. Depending on the relative gap width setting (X), ABGD identified these groupings, as well as OPHAN310 (*O. carinifera* from Macquarie Ridge) and DSOPH1855 (*O. lymani* from Patagonian shelf grouped into the South Georgia clade), implied to be two distinct species when X is set to 1. For larger values of X, ABGD failed to stabilize.

The phylogenetic method was less conservative: with the likelihood test finding the GMYC model a significant





improvement over the null model ($H_{\text{GMYC}} 505.08 \nu H_0 492.12$, $P < 0.0001$). The test identified 14 entities (CI 9–24) and 11 clusters (CI 6–16).

Gene Flow

Visual inspection of the phylogenies suggested distinct geographically distinct clades with the exception of five individuals from the Patagonian shelf that are more closely related to South Georgia haplotypes than other Patagonian haplotypes. As coalescent simulations suggest this pattern is non-random, we investigated migration between these regions.

The full model, allowing co-estimation of population size variation and free migration, indicates that migration is asymmetrical from South Georgia to the Patagonian shelf (Table 3). The posterior distribution of migration rates has clear modes: at 0.3 for migration from Patagonia to South Georgia, and 27 for South Georgia to Patagonia (Figure 6). A comparative exploration of migration scenarios strongly supports asymmetrical migration scenarios over symmetrical migration, free migration (panmixia) or no migration (Table 4). The scenario of migration from South Georgia to the Patagonian

TABLE 2 | Average pairwise distances between clades (below diagonal), associated standard error estimates (above diagonal) and within clades distances (along the diagonal).

	<i>O. lymani</i> BI	<i>O. carinifera</i>	<i>O. lymani</i> SSI	<i>O. lymani</i> PS	<i>O. lymani</i> SG
<i>O. lymani</i> BI	0.012	0.012	0.010	0.013	0.012
<i>O. carinifera</i>	0.051	0.007	0.011	0.011	0.010
<i>O. lymani</i> SSI	0.044	0.047	0.006	0.011	0.010
<i>O. lymani</i> PS	0.059	0.053	0.052	0.013	0.007
<i>O. lymani</i> SG	0.053	0.046	0.042	0.029	0.013

shelf was favored (probability of 0.59) and, although support for this model was only marginal compared with migration from the Patagonian shelf to South Georgia (probability of 0.41), it is hard to reconcile the pattern visualized in Figure 5 with migration from the Patagonian shelf as no “Patagonian shelf”-like haplotypes were found around South Georgia. This seemingly marginal result lies at least partly with the incompatibility of the distributions being compared (Figure 6). Given that the posterior distribution must be positive, and the

TABLE 3 | Posterior distributions of migration estimates from the full migration model.

	2.5%	25%	Mode	Median	Mean	75%	97.5%
SG->PS	0	9.33	27	31	33.1	39.33	74.67
PS->SG	0	0	0.33	13	19.98	12.67	71.22

The data is broken up into quantiles (25%, 75% of the distribution) and the values for the top and bottom 2.5% of the distribution is given. Of note is the large difference in the mode and the similarity of the extent of the distribution (97.5%) resulting in mean values skewed to the right.

large amount of variation expected in a single marker analysis, even a “true” value of 0 will have a wide distribution and positive mean. In this case the distribution of 97% of the data was similar for both scenarios, producing a high mean (19.98) for the PS->SG migration estimate (Table 3) even though the mode (highest posterior estimate) was near 0, compared with SG->PS where the mode (27) was much closer to the mean (33.1). Low confidence due to wide distribution of the posterior is largely down to the use of a single marker.

Timing of Divergences

The root height of the tree was estimated as 8.3 million years ago (ma) (5.77, 11.07 95% HPD). The main radiation of the *O. lymani*/*O. carinifera* clades (nodes B–D, Figure 7) was inferred to be around 2.5 ma. The most recent common ancestor of the Patagonian shelf and South Georgia shelf populations (node G) was estimated at around 1.5 ma. A similar time frame was estimated for the common ancestor of the Antarctic *O. carinifera* and its Macquarie Ridge relative (node E). The two groups of Patagonian shelf *O. lymani* that cluster within the South Georgia clade indicate that there were at least two separate migration events, one around 0.75 ma (node J) and the second around 0.22 ma (node L) although the time dependency of the molecular clock would affect these date estimates strongly and they should be considered to be more recent.

Discussion

The works of Hedgpeth (1969) and Griffiths et al. (2009) provide a biogeographic hypothesis in which the ACC strongly affect the distribution of Southern Ocean benthos by facilitating gene flow over vast geographic distances, and yet inhibiting gene flow particularly in and out of Antarctica. These processes have resulted in many species with circumpolar distributions, and assemblages that indicate a South American component, particularly at South Georgia. Our expectations under the South American input hypothesis were that there would be (1) appreciably more genetic diversity and (2) deeper coalescent times in the Patagonian Shelf populations, and (3) indication of asymmetrical movement along the direction of the ACC. However, our phylogeographic examination of *O. lymani* and *O. carinifera* demonstrated (1) very high diversity in all populations, (2) deeper coalescence times for the South Georgia population than any other, and (3) a mitochondrial signal of asymmetrical migration that appears to go against the ACC from South Georgia

to the Patagonian shelf. Importantly, it appears that there are several independently evolving lineages of *O. lymani*, with the diverging Patagonian shelf lineage breaking out of the Antarctic realm and thereby rejecting the South American input hypothesis in this case.

Crypsis

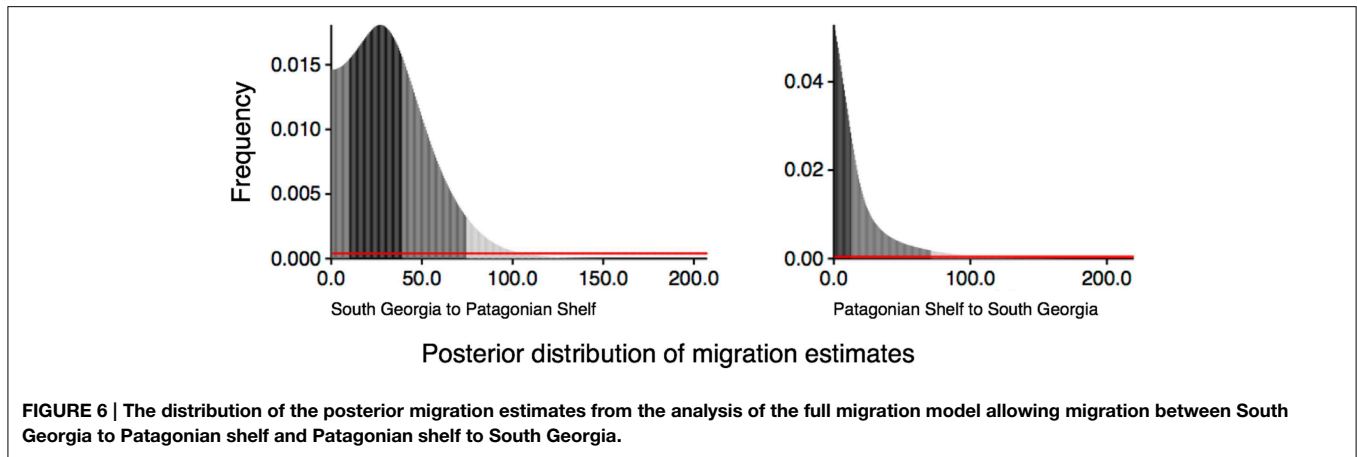
The populations of Bouvetoya and South Sandwich Islands are as genetically distinct from each other, and the Patagonian shelf and South Georgia populations, as each is also from *O. carinifera*, a clearly different morpho-species. Cryptic or unrecognized species (Janosik and Halanych, 2010) are commonly identified by mitochondrial DNA studies of benthic invertebrates (For Antarctic examples see Linse et al., 2007; Leese and Held, 2008; Krabbe et al., 2010; Arango et al., 2011; Havermans et al., 2011; Hemery et al., 2012). There is considerable debate as to whether or not biological species should be delimited based solely on mitochondrial DNA sequence data (DeSalle et al., 2005; Taylor and Harris, 2012; Zhang et al., 2013). In this case, based on clear genetic clades that correspond to discrete geographic areas, distinct morphology, and prior evidence that CO1 reliably distinguishes ophiuroid species (Ward et al., 2008), we can assume that *O. lymani* could be conservatively divided into a Bouvetoya species, a South Sandwich Island species and a diverging Patagonian shelf/South Georgia species.

Against the Flow

It appears counter intuitive that the most closely related populations are separated by what should be an intense barrier to gene flow. Similarly, despite currents that could facilitate movement from Patagonia to South Georgia, or South Georgia to the South Sandwich Islands or Bouvetoya, there is no evidence of movement in this direction or that currents themselves are responsible for population structuring. The only evidence of migration is from South Georgia to the Patagonian shelf, against the ACC and across a gradient between two water masses that strongly differ in temperature and salinity (Orsi et al., 1995).

There are three possible mechanisms for dispersal: larval transport, active adult dispersal or passive adult dispersal. There is no published literature regarding the reproductive biology of *O. lymani* or *O. carinifera*. However, lack of obvious genetic structure of *O. carinifera* haplotypes around Antarctic continental shelves and *O. lymani* haplotypes between Shag Rocks and South Georgia shelf regions indicate moderate dispersal is possible. The narrow but deep gap between South Georgia and Shag Rock shelf fragments is known to inhibit gene flow in more mobile species (Allcock et al., 1997; Strugnell et al., 2012), and yet is observed in *O. lymani*, suggesting that *O. lymani* may have a more mobile life stage. Transport of larvae in ships ballast water is a possible mechanism, although the genetic distances of the haplotypes suggest the timing of the movement substantially predates shipping in the area.

Dispersal could occur by moving down slope and along abyssal sea floor. Such a deep sea link has been used to explain the close genetic connectivity between sub-Antarctic and Antarctic morphotypes of the echinoid *Sterechinus* (Díaz et al., 2011).



The limits of adult *O. lymani/cariniifera* dispersal are unknown. They are moderately large (20+ mm disc size) and muscular, and certainly able to move across the sea floor (Sands, personal observation). Their depth range is unknown, largely due to the rarity of deep sampling events in the region. In the collections available to this study, *O. lymani* and *O. cariniifera* were only recovered in the shallower trawls (194–723 and 153–556 m depth, respectively), and absent from deeper trawls (20 trawls between 1000 and 3213 m) indicating that they prefer shelf and upper slope depths. Therefore, while dispersal of adults remains possible, it appears unlikely.

Rafting, where individuals are attached to or entangled in kelp, pumice or other buoyant substrata and drift from one area to another, is a third possible mechanism. Drifting kelp is common in the Southern Ocean (Fraser et al., 2009) and is a known dispersal mechanism for a variety of benthos (Barnes et al., 2006; Leese et al., 2010). However, rafting from South Georgia to Patagonian shelf would require either a reversal of the ACC or an almost complete circumpolar journey that, if sufficiently frequent to result in detectable gene flow, would be considerably higher in other closer shelf areas more convincingly in the path of the ACC and associated eddies, such as South Sandwich Islands and Bouvetoya.

Two other scenarios may explain the apparent migration pattern between South Georgia and Patagonia but without the need to invoke migration against the flow of the ACC. One is vicariance as a result of continental drift with retained ancestral lineages. Our results suggest that the most recent common ancestor of the clade (Node B, 2.8 ma) considerably post-dates the estimated opening of the North Scotia Ridge sufficient to allow the northward flow of the ACC around 17 Ma (Eagles, 2010), and coalescent analyses indicate that retained ancestral lineages are an unlikely explanation. A second scenario is that both populations are supplied by an un-sampled source as in the case of the limpet *Nacella magellanica*, where samples from the Atlantic South American coast and Falkland Islands show two highly divergent lineages, but also more closely related lineages and one shared haplotype. Sampling the Pacific Patagonian shelf revealed that the shared haplotype between the Atlantic population and the Falkland Islands population was the most frequent haplotype

TABLE 4 | Summary of the likelihoods of five competing migration models.

Model	Bezier lml	Bayes factor	Choice	Model Prob.
FULL	−2491.92	−13.18	3	0.00
PS->SG	−2485.71	−0.76	2	0.41
SG->PS	−2485.33	0	1	0.59
PANMIXIA	−2504.85	−39.04	4	0.00
NONE	−2585.86	−201.06	5	0.00

Log likelihoods using thermodynamic integration method ranked with a Bayes factor (BF) is determined from the Bezier method.

from the Pacific region (González-Wevar et al., 2012). We hope that further sampling opportunities will open up to test this possibility.

Out of Antarctica

A clear pattern in our data is the Antarctic or sub-Antarctic origin of the *O. lymani* / *O. cariniifera* clade. The change in morphology from *O. lymani* type to *O. cariniifera* type occurred between 2.5 and 1.6 ma, and was maintained on the Antarctic continental shelf from around 0.8 ma. Movement out of the sub Antarctic occurred between 1.4 and 0.8 ma, with two subsequent migration events 0.74 and 0.22 ma. Poulin et al. (2014) summarized the divergences of several taxa across the Drake Passage and found that divergences generally occurred around 4–5 ma, although the brittle star *Astrotoma agassizi* and the sea star *Odontaster validus* had more recent divergences of 1.5–1 ma using a 3.5% mutation rate (Hunter and Halanych, 2008; Janosik et al., 2011) which, if standardized to the mutation rate used here, would be in line with our results. Although there is some evidence of benthic groups radiating out of the Antarctic during the Tertiary (Strugnell et al., 2008; Göbbeler and Klusmann-Kolb, 2010; González-Wevar et al., 2010), and there are populations of some benthic species either side of the Polar Front (see Supplementary Tables in Sands et al., 2013), to our knowledge this is the first evidence strongly suggesting Quaternary asymmetrical movement *out* of the Antarctic and into warmer waters. At a time when regional warming is causing a detectable shift in range distributions toward the poles (Thomas, 2010), and there is an expectation that

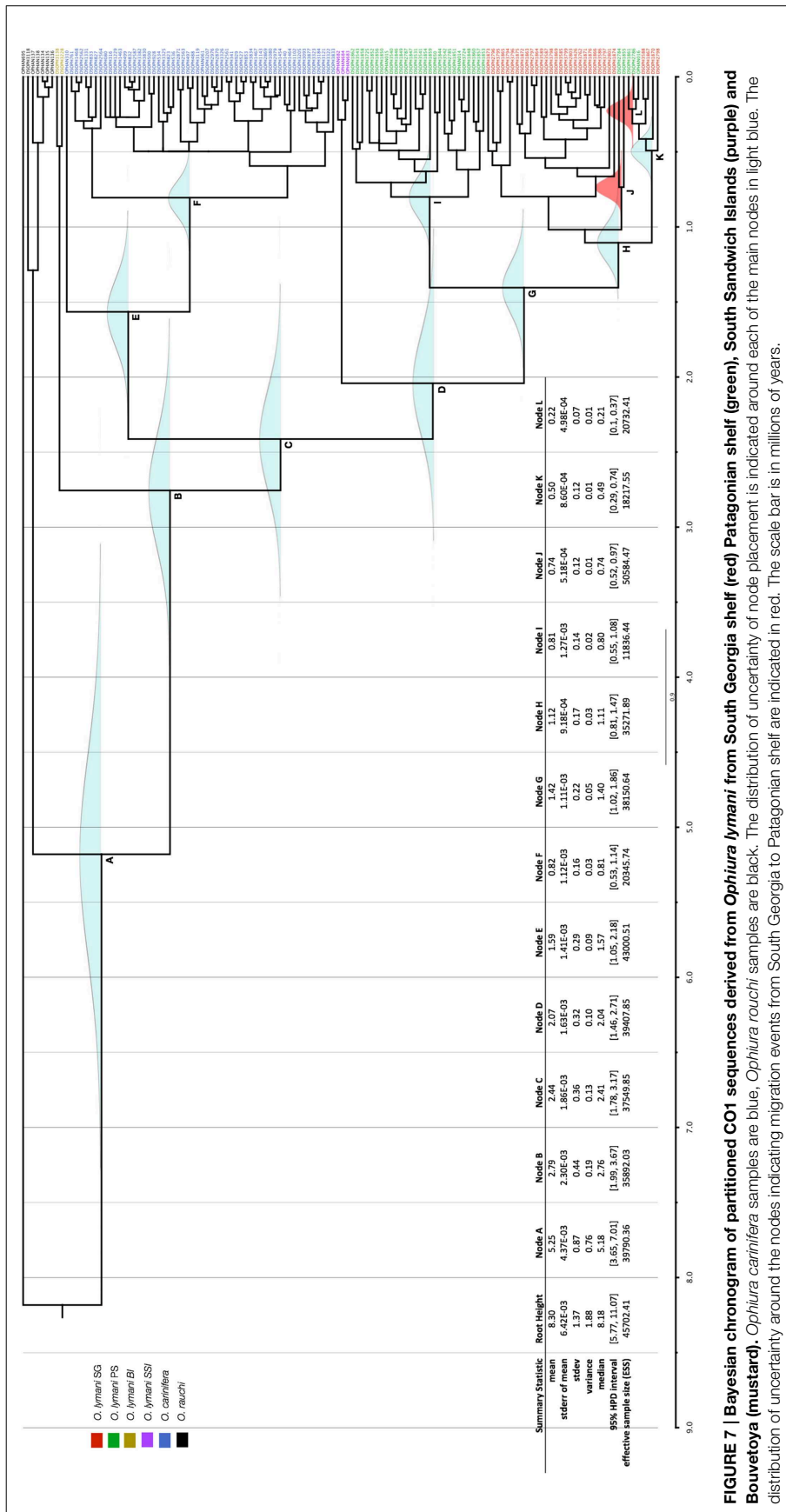


FIGURE 7 | Bayesian chronogram of partitioned CO1 sequences derived from *Ophiura lymani* from South Georgia shelf (red) Patagonian shelf (green), South Sandwich Islands (purple) and Bouvetoya (mustard). *Ophiura carrillera* samples are blue, *Ophiura rauchi* samples are black. The distribution of uncertainty of node placement is indicated around each of the main nodes in light blue. The distribution of uncertainty around the nodes indicating migration events from South Georgia to Patagonian shelf are indicated in red. The scale bar is in millions of years.

indigenous Antarctic cold-adapted species may be threatened by the warming marine environment (Peck, 2005), the example of *O. lymani* shows that at least one species has had the ability to migrate and adapt to warmer temperature regimes over time.

Caveats and Interpretation Limits

Although evidence for cypsis, asymmetrical migration and radiation out of Antarctica appears compelling, there are caveats that apply to the current results and interpretations. The DNA sequence marker cytochrome c oxidase subunit 1 is widely used and heavily relied on in species identification and biodiversity studies. However, we emphasize that it is a single line of evidence and despite our best attempts at controlling for stochastic or confounding processes, without other gene sequences we cannot be overly confident that our gene trees reflect the species tree. Similarly, our mutation rates are best estimates, and the process they are derived from (the closing of the Isthmus of Panama) is in itself a best estimate, so the dates and error distributions provided here should be treated with great caution. The analyses here assume mutation drift equilibrium and selective neutrality, neither of which are safe assumptions in real populations. Finally, the sampling, although the best available, is still limited due to the enormous time and expense of carrying out surveys in the Southern Ocean and remote southern areas of other oceans. Perhaps the most significant sample related limitation for this study is the geopolitical boundaries on the South American continent, which have prevented wide sampling of the Patagonian shelf. However, with these caveats in mind, we can still interpret clear patterns of diversification within the Southern Ocean and what appears to be a recent movement from of lineages from South Georgia to the Patagonian Shelf.

Conclusions

We have found evidence that the ophiuroid *O. lymani* has radiated in the Southern Ocean, with deep sea between isolated lineages likely to be the mechanism maintaining distinct populations or cryptic species. Our results indicate occasional migration over evolutionary time against the prevailing ACC

and across the perceived barrier of the Polar Front. We provide evidence that *O. lymani* is a species complex of which *O. carinifera* is an element, with the South Georgia population highly divergent from other geographic populations in the Southern Ocean, and the likely source of an invasion into the cool temperate seas of South America. These results and hypotheses may cause a rethink in our general view of Antarctic biogeography, but will require further testing including better South American sampling, replication of markers, and extension to other taxa.

Author Contributions

CS conceived the study, collected the majority of the specimens, acquired funding for the molecular sequencing, conducted the analyses and wrote the manuscript. TO provided additional specimens and sequences, helped shape the manuscript. DB assisted in the collections both financially and practically, contributed to the text and provided intellectual input throughout the project. RL identified the majority of the specimens, provided unique and relevant observations and contributed to the production of the manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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