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Title: Trans-Arctic speciation of Florideophyceae (Rhodophyta) since the opening of the Bering Strait, with consideration of the “species pump” hypothesis

Short title: Trans-Arctic speciation in Rhodophyta

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Abstract

Aim: The opening of the Bering Strait initiated significant biotic interchange that is postulated to have played a major role in phylogeographic patterns of northern marine flora and fauna. In addition, the “species pump” hypothesis asserts that glaciation events promoted speciation due to repeated isolation of populations over the past 2.6 million years. Here, trans-Arctic speciation events in red marine macroalgae (Florideophyceae) were assessed using time-calibrated phylogenies, and the applicability of the “species pump” hypothesis was considered.

Location: Species records and sequence data for trans-Arctic genera of marine macroalgae were amalgamated and supplemented with sampling from the Northwest Atlantic, Northern Alaska (Beaufort Sea), Norway, and Nome, Alaska (Bering Sea; 2014-2017).

Methods: Bayesian and maximum-likelihood phylogenies were variously built using the 5’ end of the cytochrome *c* oxidase subunit I gene (COI-5P), and/or the full length nuclear internal transcribed spacer region (ITS), and/or the ribulose-1 5-biphosphate carboxylase large subunit gene (*rbcL*), and nodes were timed using calibrated COI-5P and *rbcL* molecular clocks. The final dataset represented approximately 184 species, broadly representing 14 trans-Arctic lineages.

Results: Pacific to Atlantic migration and subsequent speciation was inferred in 11 cases, whereas the opposite scenario, Atlantic to Pacific, was inferred once; only three speciation events appeared to occur during the Pleistocene.

Main conclusions: Our results are in agreement with previous studies in that trans-Arctic speciation events post-dated the opening of the Bering Strait with a clear Pacific to Atlantic bias. Evidence for the “species pump” (as applied to trans-Arctic interchange) was lacking given the frequency of trans-Arctic speciation events was not amplified during the Pleistocene.

Keywords: Macroalgae, marine, glaciation, Pleistocene, molecular clock, dispersal

Introduction

Glaciation events during the Pleistocene are hypothesized to have driven allopatric speciation events due to repeated isolation of populations into glacial refugia, a mechanism originally proposed by Haffer (1969) called the “species pump.” This hypothesis was intended to explain Amazonian bird diversity, though the “species pump” hypothesis for Neotropical settings has since undergone scrutiny (Knapp & Mallet, 2003). Nonetheless, investigations into the species pump sensu Haffer (1969) in other settings have been conducted, and have generally produced favorable evidence, including results from alpine habitats (Schoville *et al.*, 2012), freshwater systems (April *et al.*, 2013), and marine Antarctica (Clarke & Crame, 1992; Wilson *et al.*, 2009), although results for invertebrates on island systems do not appear to support this hypothesis (Papadopoulou & Knowles, 2015, 2017). The “species pump” hypothesis may also apply to vicariance of conspecifics in the North Atlantic and North Pacific during Pleistocene glacial periods, facilitated by repeated openings of the Bering Strait. Atlantic and Arctic marine invertebrate taxa are believed to have initially migrated into the Pacific basin upon the opening of the Bering Strait 4.8-7.4 million years (Ma) ago (Marincovich & Gladenkov 1999; Marincovich 2000). Changes to ocean circulation patterns, mediated by the formation of the Isthmus of Panama (Haug & Tiedemann, 1998; Raymo, 1994), subsequently caused Pacific species to flow into the Atlantic, a pattern again documented in invertebrate taxa (Briggs, 1970; Reid, 1990; Vermeij, 1991). Recent genetic studies in various taxa have confirmed the presence of sister lineages on either side of the North American Arctic, further suggesting interchange between the Pacific and Atlantic basins has driven speciation, or at the very least, divergent populations (cod: Bigg *et al.*, 2008, and references therein; seagrass *Zostera marina* Linnaeus: Olsen *et al.*, 2004; *Macoma balthica* Linnaeus complex: Nikula *et al.*, 2007; echinoderms and polychaetes: Carr *et al.*, 2011; Hardy *et al.*, 2011; Loeza-Quintana & Adamowicz, 2018).

Marine macroalgae are an ideal group for investigating speciation patterns related to the opening of the Bering Strait. Dispersal across the Arctic is likely facilitated by asexual reproduction and heteromorphic generations that may tolerate a wide range of environmental conditions, easing the establishment of flora in new environments (Graham & Wilcox, 2000). As well, in some cases, buoyant species of marine macroalgae may disperse large oceanic distances, potentially rafting associated flora and fauna (Fraser *et al.*, 2011; Saunders, 2014; Macaya *et al.*, 2016). As such, the opening of the Bering Strait is expected to have left phylogenetic signatures

in a variety of marine macroalgae, signatures that are possibly mirrored in the fauna utilizing macroalgae as habitat (Christie *et al.*, 2009). Investigations of trans-Arctic interchange on phylogeographic patterns in marine macroalgae have demonstrated a strong Pacific to Atlantic migration pattern. Sister lineages of marine macroalgae in the North Pacific and North Atlantic have been recognized for some time (Lindstrom, 1987), with trans-Arctic interchange and speciation inferred from patterns in phylogenies (Lindstrom, 2001). Notable Atlantic species that are hypothesized to have resulted from a Pacific ancestor include *Chondrus crispus* Stackhouse, *Dilsea carnosa* (Schmidel) Kuntze, *Dumontia contorta* (S.G.Gmelin) Ruprecht, and *Phycodrys rubens* (Linnaeus) Batters (Lindstrom, 2001). Atlantic to Pacific migrants noted by Lindstrom (2001) represented conspecifics (e.g. *Coccotylus truncatus* [Pallas] M.J.Wynne & J.N.Heine and *Fucus distichus* Linnaeus). In contrast, Coyer *et al.* (2011) hypothesized *F. distichus* colonized the Atlantic from the Pacific on two occasions, however, recent analyses indicate that this species may have a uniquely Arctic origin for both Pacific and Atlantic species/sub-populations (Laughinghouse *et al.*, 2015). Species of *Porphyra* in Greenland ally closely with species in the North Pacific (Mols-Mortensen *et al.*, 2014), while *Laminaria* spp. are hypothesized to have migrated into the Atlantic twice since the opening of the Bering Strait (Rothman *et al.*, 2017). The above speciation scenarios are further supported by thermogeographic modeling that indicates a strong Pacific to Atlantic connection exists in marine macroalgae, which played a critical role in the establishment of the Arctic and North Atlantic flora during the Pleistocene (Adey & Hayek, 2011; Adey *et al.*, 2008). Vicariant trans-Arctic populations also appear to be a common feature in the Arctic marine flora, suggesting trans-Arctic migrations continue to play a role in promoting biological diversity (Saunders & McDevit, 2013).

Despite the above evidence, it remains unclear whether the “species pump” is applicable to trans-Arctic lineages of marine macroalgae. For one, the studies cited above interpret migration and speciation through the Bering Strait post-factum to explain phylogeographic patterns. Indeed, the opening of the Bering Strait is used as a calibration point for the Atlantic clade (excluding *Laminaria solidungula* J.Agardh) in the Rothman *et al.* (2017) study on *Laminaria* (although time points calibrated using fossil evidence are also used). An independently calibrated molecular clock testing divergence time estimates in trans-Arctic lineages is needed to assess whether these events occurred after the opening of the Bering Strait, and if these events took place during glaciation. In particular, the “species pump” hypothesis

asserts that Pleistocene glaciation precipitated speciation events by repeatedly pushing populations into refugia. Many of the speciation events cited above may be the result of the initial opening of the Bering Strait, and not precipitated or amplified directly through glaciation events *per se*. These processes could be teased apart by examining whether or not the number of trans-Arctic speciation events (i.e. migration across the Arctic followed by vicariance) appear to increase or are more common during the Pleistocene (2.6 Ma-12 ka; Miller *et al.*, 2010) as compared to the estimated time frame between the opening of the Bering Strait and the Pleistocene (7.4-2.6 Ma; Marincovich & Gladenkov, 1999).

Given the above uncertainties, we sought to determine if time-calibrated phylogenetic trees are consistent with trans-Arctic interchange of red marine macroalgae and considered the applicability of the “species pump” hypothesis. Our research questions were: 1) do time calibrated phylogenies support the view that marine macroalgae have undergone trans-Arctic migration events since the opening of the Bering Strait, primarily from the Pacific to the Atlantic? And if so, 2) did these speciation events primarily occur during the Pleistocene, in support of the “species pump” hypothesis? This investigation will yield insight on recent evolutionary events in the Northern Hemisphere and add to the understanding of biotic interchange through the Bering Strait and its impacts on contemporary biogeographic distributions in marine coastal systems. Understanding historical migration pathways in Arctic marine species is urgently needed as biotic interchange across the Arctic basin is expected to increase in the wake of climate change (Vermeij & Roopnarine, 2008). Furthermore, updated phylogenies assessing trans-Arctic speciation events are timely given the surge in genetic data since the last review of this topic nearly two decades ago (Lindstrom, 2001).

Materials and Methods

Data collection

To assess if trans-Arctic migrations have resulted in speciation events during the Pleistocene, we amalgamated preexisting records for three markers (5’ end of the cytochrome *c* oxidase subunit I gene [COI-5P], the full length nuclear internal transcribed spacer region [ITS], and the ribulose-1 5-biphosphate carboxylase large subunit gene [*rbcL*]) in targeted macroalgal

species from the Barcode of Life Data Systems and GenBank, and supplemented our dataset with collections from Baffin Island, through northern Labrador to Makkovik (Canada; September 2-12, 2014); the Beaufort Sea, Northern Alaska (USA; August 20-21, 2014, and August 20-25, 2015); Bergen and surrounding area (Norway; June 3-13, 2016); and Nome, Alaska, and surrounding area (Northern Bering Sea, USA; August 12-22nd, 2017). Specimens were collected from the intertidal or subtidally via scuba. Vouchers were taken as small pieces of algal frond tissue preserved in silica, or pressed and dried, and/or as electronic images. A 1 cm² portion of tissue was also preserved in silica and brought back to the University of New Brunswick for DNA extraction using a QIAGEN TissueLyser II and QIAxtractor robot for high throughput protocols, as used by Saunders and McDevit (2012a). Primers and thermocycling regimes are listed in Table 1. Amplified PCR products were sent to Genome Quebec for forward and reverse sequencing. Sequences were edited and aligned in Geneious v.10 (Kearse *et al.*, 2012) for each species. For a specimen list with GenBank accession numbers, see Table S1 in Supporting Information.

Molecular clock calibrations and phylogenetic analyses

Phylogenetic trees were separately built for each trans-Arctic lineage of red macroalgae, and nodes were dated using calibrated COI-5P and *rbcL* clocks in BEAST v.1.8.3 (Drummond *et al.*, 2012). Molecular clocks were applied to the coding markers rather than ITS (non-coding) given these former evolve at relatively predictable rates (i.e. rare insertions or deletions, and primarily synonymous substitutions). The same molecular clocks in COI-5P and/or *rbcL* were applied in independent analyses of each lineage. In order to accommodate rate heterogeneity across lineages, a range of clock rates were applied as priors in the Bayesian analyses, and the strict clock model was loosened in follow up analyses where rate heterogeneity was detected within a given lineage. Divergence time estimates (proxied through mean node heights) were derived from the updated analyses wherein the molecular clock constraints were loosened. Trans-Arctic genera were included in the analyses based on previous DNA barcoding and taxonomic work (genera previously targeted for taxonomic work in the North Atlantic and North Pacific are likely to have most of their genetic groups sampled across a broad geographic range and multiple genetic markers sequenced, typically COI-5P and *rbcL*); less well-studied taxa, such as red crusts, were excluded from the analyses. As such, all geographic ranges in all species

are based on genetically verified records. The following genera of interest were used to build 14 phylogenetic trees: from Ahnfeltiales *Ahnfeltia*; from Palmariales *Devaleraea*, *Halosaccion*, and *Palmaria*; from Gigartinales *Coccotylus*, *Phyllophora*, and *Ozophora*; *Dilsea*, *Hyalosiphon*, and *Neodilsea*; *Mastocarpus*; *Opuntiella* and *Turnerella*; from Ceramiales *Melanothamnus*; *Membranoptera*; *Odonthalia*, *Neorhodomela*, and *Rhodomela*; *Polysiphonia*; *Phycodrys*; *Polyostea*; *Ptilota*; *Vertebrata*.

The COI-5P clock was calibrated by analyzing trans-Atlantic sequences of *Palmaria palmata* (Linnaeus) F.Weber & D.Mohr (20 mitotypes, 661 bp) with an estimated isolation time between Northeast and Northwest Atlantic populations of 0.906 Ma, which was estimated using *cox2-3* and plastid *rpl-rps* markers (Li *et al.*, 2015). Owing to an error by Li. *et al.* (2015), who confused sequence divergence with substitution rate, we had to double the isolation times reported by them. These markers were initially calibrated using divergence estimates in *Bostrychia calliptera* (Montagne) Montagne and *Bostrychia pinnata* J.Tanaka & Chihara, and a calibration point of the formation of the Isthmus of Panama 3-3.5 Ma (Zuccarello & West, 2002; Coates & Stallard, 2013). BEAST runs consisted of the following parameters: empirical base frequencies; an HKY substitution model; a strict clock; a Yule speciation tree prior; a random starting tree; priors on the root height of the tree set to a normal distribution with a mean of 0.906 and a normal distribution truncated around 0.696 and 1.432 (as per pairwise populations min and max isolation time estimates in Li *et al.* [2015]), and a standard deviation of 0.1; and otherwise uninformative (e.g. uniform) priors. Three 10 million length chains were run with sampling every 1000 steps, for a total of 30,000 genealogies saved. Results were combined and assessed after a burn-in of 1000 genealogies in Tracer v.1.6.0 (Rambaut *et al.*, 2014). The combined runs resulted in an HKY COI-5P substitution rate of 0.00760 substitutions/site/Ma (with 95% Highest Posterior Density [HPD] min and max estimates of 0.00352 and 0.01205 substitutions/site/Ma). The above parameters were used to build a first round of phylogenetic trees in each trans-Arctic lineage assessed, using a COI-5P clock rate prior of 0.00760, with a normal distribution and a standard deviation of 0.005 (approximating the min and max clock estimates of 0.00352 and 0.01205), in addition to unlinked HKY substitution and strict clock models across markers and partitioning of three codon positions (except in the case of ITS); parameters for the analyses were otherwise the same as above for the calibration of COI-5P.

The molecular clock assumption was tested on the initial phylogenetic trees using Maximum Likelihood (ML) tests under the Tamura-Nei model in MEGA v.7.0.26 (see Table S2 in Supporting Information; Tamura and Nei, 1993; Kumar *et al.*, 2016). In some cases, a subset of the initial tree for a given lineage was tested for the molecular clock hypothesis (see Table S2). Tajima's Relative Rate Test was also used to test the molecular clock hypothesis in *Ahnfeltia* and *Opuntiella*/*Turnerella* (see Table S3 in Supporting Information; Tajima, 1993; Kumar *et al.*, 2016). A second round of phylogenetic analyses were then conducted wherein the molecular clock constraint was loosened based on the results from ML and Relative Rate tests (see Table S4 in Supporting Information). This process began by loosening the molecular clock assumption for *rbcL* in lineages where a uniform rate of *rbcL* evolution was rejected, using an uncorrelated relaxed clock model (Drummond *et al.*, 2006), and enforcing a strict COI-5P clock using the same priors as in the initial analyses (see Table S4). We also reanalyzed a subset of taxa in some lineages wherein the molecular clock was not rejected for COI-5P and *rbcL* (see Table S2), again enforcing strict clocks and the same priors as in the initial analyses. The average global *rbcL* rate from these analyses (0.00301 substitutions/site/Ma, with average 95% HPD min and max estimates of 0.00142 and 0.00453; see Supporting Information Table S5) was then used in lineages for which the molecular clock hypothesis was rejected for COI-5P; a strict *rbcL* clock with a normal distribution approximating the above clock range was employed in these analyses, while a random local clock was used for COI-5P (see Table S4). Uncorrelated relaxed clocks for COI-5P and *rbcL* were used in *Polyostea* and *Odonthalia*, setting priors on the global COI-5P and *rbcL* clocks to the above estimated rates for each marker. Finally, Relative Rate Tests detected rate heterogeneity in *Turnerella* sp. 1BC in the analysis of *Opuntiella*/*Turnerella* (see Table S3). We therefore reanalyzed this lineage without *T. sp. 1BC*, using a strict *rbcL* clock.

In addition to the BEAST analyses, tree topology and node support were assessed by building ML trees using the RaxML v7.2.8 plugin in Geneious v.10 (Stamatakis, 2006), utilizing an HKY substitution model, and partitioning the data according to marker and codon position. Saturation was assessed for COI-5P and *rbcL* for each lineage by plotting the number of transitions and transversions against F84 genetic distances between pairs of sequences using DAMBE6 (Xia, 2017). Outgroups were not included in these analyses. Saturation was visually assessed, and defined as the approximate genetic distance at which the number of transversions

notably increased relative to the number of transitions (see Supporting Information Figs. S1 and S2). For ML trees see Supporting Information Figs. S3-S22.

Interpretation of phylogenetic trees

Trans-Arctic speciation events were inferred according the following criteria: a given species or clade had to be embedded in a clade of species from the opposite basin (e.g. an Atlantic species embedded in a clade of Pacific species would imply a trans-Arctic speciation event wherein a Pacific ancestor migrated into the Atlantic basin and subsequently speciated); node support had to be ≥ 0.95 posterior probability for a given time-calibrated node (although poorly supported nodes were used to approximate divergence times in *Polysiphonia* and an earlier event in *Dilsea*; inconsistencies between Bayesian and ML trees are discussed in the Supporting Information); and divergence time estimates had to fall within the past 7.4 Ma, given this is the oldest date the Bering Strait is hypothesized to have opened (Marincovich & Gladenkov, 1999).

Results

In total, records were amalgamated from 289 specimens, 55 of which were collected during the course of this study (2014-2018; see Table S1). Approximately 184 species were analyzed, broadly representing 14 trans-Arctic lineages (Figs. 1 and 2). Overall, Pacific-to-Atlantic migration and subsequent speciation since the opening of the Bering Strait was inferred in 11 cases (not including *Odonthalia dentata*, see Supporting Information), whereas the opposite scenario, Atlantic-to-Pacific, was inferred in one; the direction of a further two cases of trans-Arctic migration and speciation remained uncertain (Fig. 3). Regarding the timeframe for these events, speciation during the Pleistocene was inferred in three cases, while another eight events appeared to occur after the opening of the Bering Strait but prior to the Pleistocene; a trans-Arctic speciation event in both *Ahnfeltia* and *Odonthalia* may fall into either time frame (Fig. 3). Specific findings for each trans-Arctic lineage are summarized in the Supporting Information. Species hypothesized to have descended from a trans-Arctic migration and speciation event either featured contemporary Arctic populations, or were otherwise closely related to a species or lineage with Arctic populations (bolded species in Figs. 1 and 2), the notable exception being *Melanothamnus*. In addition, trans-Arctic species (i.e. species with

contemporary populations in the Atlantic and the Pacific) occurred in half of the lineages examined.

Discussion

Trans-Arctic interchange of species since the opening of the Bering Strait is believed to have played a major role in the establishment of current day northern marine flora and fauna. In addition, the “species pump” hypothesis asserts that Pleistocene glaciation promoted speciation by repeatedly isolating populations over the past 2.6 Ma. Here, we have attempted to confirm the timing of putative trans-Arctic speciation events in marine red macroalgae to assess whether these events post-date the opening of the Bering Strait, and if these events are notably greater in frequency during the Pleistocene in support of the “species pump” hypothesis.

Opening of the Bering Strait and “Pacification” of the Arctic/Atlantic

Time-calibrated phylogenies are in agreement with previous studies that suggest dispersal through the Bering Strait has played an important role for speciation in several trans-Arctic lineages of marine macroalgae. For instance, the ancestor to *Palmaria palmata* is hypothesized to have crossed the North American Arctic following the opening of the Bering Strait (Lindstrom *et al.*, 1996), which is in close agreement with our time calibrated phylogeny for Palmariales (Fig. 1B). Similar hypotheses have been put forward to explain the presence of *Dilsea* and *Phycodrys* in the North Atlantic (Lindstrom, 2001; van Oppen *et al.*, 1995), which are also in agreement with the time calibrated phylogenies presented here (Figs. 1D and 2E). In addition, nearly all species resulting from a putative trans-Arctic speciation event either feature Arctic populations or are closely related to a species or lineage with contemporary Arctic populations (Figs. 1 and 2), further supporting the hypothesis that an ancestor crossed the Arctic upon the opening of the Bering Strait. Lindstrom (1987) emphasized the importance of biogeographic tracks (e.g. consistent results between lineages) to establishing whether or not dispersal through the Bering Strait played a critical role in establishing the Arctic and temperate North Atlantic floras. Thirty years later, these tracks have been sufficiently laid to confidently assert the opening of the Bering Strait represented a major biogeographic event in the establishment of contemporary Arctic and Northwest Atlantic marine macroalgae (Fig. 3). A similar history of biotic interchange between the Atlantic and Pacific through the Bering Strait is expected to have played a significant role in structuring biogeographic distributions in other marine taxa. Indeed,

similar results have already emerged in other marine flora (seagrass *Zostera marina*: Olsen *et al.*, 2004) and fauna (cod: Bigg *et al.*, 2008; echinoderms and polychaetes: Carr *et al.*, 2011; Hardy *et al.*, 2011). The opening of the Bering Strait therefore appears to have played a significant role in structuring contemporary boreal and temperate marine coastal communities.

These results further support the view that Pacific to Atlantic speciation events were far more common than Atlantic to Pacific events. The results presented here indicated that Pacific to Atlantic speciations following the opening of the Bering Strait occurred at a rate of 11:1 compared to Atlantic to Pacific speciations (Fig. 3). This Pacific to Atlantic bias has remained practically unchanged since the beginning of investigations on the Bering Strait and its impact on the phylogeographic history of Northern flora and fauna (Briggs, 1970; Vermeij, 1978; Lindstrom, 1987, 2001; Reid, 1990; Coyer *et al.*, 2011, and references therein). Several explanations have been put forward to explain this bias. For one, the predominant motion of water has been west to east through the Arctic for approximately the past 4.6 Ma (Haug & Tiedemann, 1998), which may have enhanced asymmetric dispersal pathways in marine macroalgae through the Arctic. Vermeij (1991) favoured an ecological opportunity hypothesis to explain marine mollusk distributions that similarly displayed a Pacific bias in Northwest Atlantic fauna. This hypothesis asserts that invaders are proportional to extinction rates in a region; if true, it may be that ecological niches were regularly left vacant in the Northwest Atlantic due to relatively harsher glaciation conditions, facilitating the establishment of Pacific algal and animal species. More recently, thermogeographic modelling has indicated that Arctic and Atlantic sub-Arctic species have continually been derived from the North Pacific due to Pleistocene climate and geography (Adey *et al.*, 2008; Adey & Hayek, 2011). A combination of factors likely played a role in the establishment of Pacific lineages in the Atlantic since the opening of the Bering Strait, and phylogeographers will undoubtedly continue to unravel these processes.

Impediments to the “species pump” hypothesis

Dispersal through the Bering Strait may have played an important role in diversification of Arctic and North Atlantic flora, but the “species pump” sensu Haffer (1969) did not find support in our analyses. Speciation events did occur during the Pleistocene, however, there was no obvious bias for trans-Arctic speciation events to have occurred during this time (Fig. 3). In other words, speciation through the Bering Strait in the absence of glaciation appears to be just

as common as speciation during the Pleistocene (if the number of events is standardized by the length of the timeframes). This implies that speciation facilitated by an opened Bering Strait is a sufficient explanation for the phylogeographic patterns reported here, with the glaciation-driven “species pump” offering little additional explanatory power. In particular, multiple trans-Arctic speciation events within a genus are notably absent during the Pleistocene. Though this is not a criterion for the “species pump” (as this hypothesis applies to the potential for species diversity to further increase), the lack of an amplifying effect to speciation during the Pleistocene suggests populations were not regularly isolated on either side of the Bering Strait, or were not isolated for long enough periods of time to develop reproductive isolation.

We hypothesize that the “species pump” is inhibited by 1) niche occupation by species/lineages adapted to Arctic conditions, preventing recurrent trans-Arctic events, and 2) secondary contact between Arctic adapted lineages/species during interglacial periods. The progression of events starting with a Pacific lineage may have followed this trajectory: a temperate Pacific ancestor gradually became adapted to northern conditions (i.e. extremes in seasonality, cold-water, ice-cover in some cases beginning in the Pleistocene); this north-adapted ancestor eventually traversed the Arctic, providing the Bering Strait was open; the Arctic lineage was maintained (species bolded in Figs. 1 and 2), while some populations moved south into temperate Atlantic waters; these southerly populations gave rise to contemporary Atlantic species descended from a Pacific ancestor. Given this scenario, the “Arctic” niche remains occupied by the lineage/ancestor that facilitated an initial crossing of the Arctic upon the opening of the Bering Strait, preventing sister lineages from moving north and initiating new trans-Arctic migrations/speciation events. If the above scenario holds true, then the “species pump” is acting on north-adapted species/lineages, pushing populations into refugia on either side of the Arctic during glaciation (i.e. North Atlantic and the North Pacific). Secondary contact during interglacial periods, however, may have inhibited the speciation process. This is suggested by the large portion of lineages analysed here with trans-Arctic species ($n=7/14$, not including potential introductions; Figs. 1 and 2), indicating dispersal across the North American Arctic is common in contemporary north-adapted populations. Furthermore, secondary contact in the Canadian Arctic by vicariant populations is evident in *Saccharina latissima* (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders (McDevit & Saunders, 2010; Neiva *et al.*, 2017) and in *Scagelia pylaisaei* (Montagne) M.J.Wynne (Bruce pers. comms.). In another study assessing the

applicability of the “species pump,” increased gene flow during glaciation in island systems (due to land bridges) was forwarded as a mechanism preventing vicariant speciation in a species of cricket (Papadopoulou & Knowles, 2015). The dilution of divergence through secondary contact may be further facilitated by a slow rate of evolution in marine macroalgae, as suggested by the substitution rates estimated here (approximately 0.7%/Ma for COI-5P, see methods). LeGall and Saunders (2010) found COI-5P to exhibit, typically, 1% or more divergence between species. This means populations likely require several hundreds of thousands of years of isolation before becoming reproductively isolated, which greatly exceeds the time scale for cycles of glaciation (41-100 ka; Lisiecki & Raymo, 2005; Miller *et al.*, 2010). This could be particularly true for brown macroalgae, as they appear to accumulate substitutions slower than red lineages (Bringloe & Saunders, 2018; Smith, 2015).

In sum, support for the “species pump” is weak as applied to trans-Arctic lineages of marine macroalgae. Unoccupied niches in the North Atlantic were likely filled by Pacific lineages following the opening of the Bering Strait, leading to long term establishment and eventual speciation. Subsequent population divergence across the Arctic due to glaciation events was possibly ephemeral as evolution was slow and secondary contact in north-adapted species was apparently common during interglacial periods. The “species pump” (as applied here) therefore appears to have contributed little to contemporary species diversity, though the role of Pleistocene glaciation in structuring contemporary populations warrants further investigation. Population level datasets in trans-Arctic species of marine macroalgae are needed to confirm if admixture is common in the Canadian Arctic, and will additionally help resolve some of the speciation scenarios outline above (e.g. *Ahnfeltia*, *Coccotylus*, *Dilsea*, *Odonthalia*, *Phycodrys*, *Rhodomela*).

Limitations in inferring phylogenetic events

The use of molecular clocks is not without shortcomings, some of which have the potential to impact interpretation of phylogenetic trees (Heads, 2005; Lessios, 2008). A critical caveat here is that we have assumed that the initial calibration point (the closing of the Isthmus of Panama) corresponds in time with the divergence of *Bostrychia calliptera* and *Bostrychia pinnata* (Zuccarello & West, 2002), however, divergence between these species may have occurred earlier in time. If this speciation event indeed occurred earlier, then the molecular clock

rates inferred in our study will be overestimated. Slower clock rates would lead to earlier divergence time estimates in our phylogenies, further placing the inferred speciation events prior to the Pleistocene and bolstering our conclusions regarding the “species pump” hypothesis (see below). Another important consideration regarding the use of molecular clocks is rate heterogeneity amongst the lineages analyzed. Indeed, the range in molecular clocks applied here across a broad range of taxa (i.e. Florideophyceae) will undoubtedly be inaccurate in some cases (saturation estimates suggested the rate of COI-5P evolution in Ceramiales was considerably higher than in the Gigartinales; see Fig. S1). We attempted to address molecular clock assumptions by relaxing this constraint where rate heterogeneity was detected, however, the original rates invoked in the analyses remain axiomatic to orienting the interpretations of the phylogenies in time. On a positive note, our timed phylogenies appeared to accommodate independent geological time events (e.g. possible movement of an ancestor through the Proto-Caribbean Seaway in *Melanothamnus*; Fig. 3), and were consistent with previously hypothesized scenarios of dispersal through the Bering Strait (see discussion below), suggesting the rates invoked were within the realm of possibility.

The inferences made here also rest on the critical assumption that phylogenetic patterns should be interpreted in such a way that minimizes the number of trans-Arctic speciation events. There is, however, precedence to interpret genetic patterns within a species or species complex as the result of recurrent Pacific to Atlantic migration events in both macroalgae and invertebrate taxa (Nikula *et al.*, 2007; Coyer *et al.*, 2011), which would appear as cascading speciation events in a phylogenetic tree (e.g. an Atlantic species/ancestor seeding multiple Pacific lineages through time). Speciation within an oceanic basin (as opposed to recurrent trans-Arctic events) was given preference in interpreting the phylogenetic patterns presented here given 1) the impediments to dispersal presented by the Arctic (ice-scour, patchy rocky habitat, and extreme seasonality), at least for the majority of species analyzed (which were generally temperate); and 2) the greater number of avenues for allopatric speciation in the Atlantic and Pacific (east-west and north-south distributions) versus the Arctic (east-west distributions exclusively). More complex scenarios (involving more than one migration event across the Arctic, or sympatric speciation followed by migration and extinction of a given species in the parental basin), nonetheless, could have occurred (e.g. *Phycodrys*; see Supporting Information). In addition, the scenarios presented here are certainly incomplete, as now extinct species may have played an important role. The number

of pre-Pleistocene speciation events may be underestimated since the Bering Strait opened during an earlier timeframe, several millions of years prior to the Pleistocene. As such, conclusions regarding the species pump, if anything, are bolstered by this shortcoming. Non-sampled extant species, similarly, have the potential to change interpretations of the phylogenies. Improvements to the results presented here, such as the calibration of alternate markers or improved data resolution through improved geographic coverage and genomic level datasets (e.g. Jackson *et al.*, 2017), are eagerly anticipated.

Conclusions

The role of the Bering Strait and biotic interchange between the Pacific and the Atlantic has captured the attention of biologists for nearly half a century (Briggs, 1970; Lindstrom, 1987, 2001), and our understanding of this phenomenon has certainly improved during this time. Here, time calibrated phylogenies confirmed that Pacific to Atlantic speciation events post-dating the opening of the Bering Strait were common in trans-Arctic lineages of marine macroalgae. Despite this, the “species pump” hypothesis does not appear to be applicable during the Pleistocene given the lack of amplification in species diversity. The putative slow rate of evolution in marine macroalgae and hypothesized regularity of secondary contact between divergent trans-Arctic lineages likely inhibit the speciation process. While Pleistocene glaciation certainly played a major role in structuring contemporary populations of marine macroalgae, its role has likely been limited to the emergence of these species.

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Biosketches

Trevor T Bringle is broadly interested in biogeographic patterns in Arctic marine flora. This work represents a component of his PhD work at the University of New Brunswick on historical and contemporary origins of North American Arctic marine flora.

Gary W Saunders has spent his career using integrative taxonomy (molecular and morphological) to resolve phylogenetic relationships and taxonomic issues in marine macroalgae.

Author contributions: TTB and GWS conceived the ideas; TTB and GWS conducted the field work; TTB conducted the lab work and data analyses; TTB led the writing with the assistance of GWS.

Table 1. Primers used for amplification and sequencing of various genes in marine macroalgae. Primer sequence portions in bold for M13LF3 and M13Rx forward and reverse primers, respectively, refer to sequencing primers used (M13F and M13R).

Locus	Taxa	Primers	Primer sequence (5'-3')	Reference/thermocycling regime
COI-5P	Red algae	M13LF3	TGTAAAACGACGGCCAGT ACHAAYC AYAARGATATHGG	Saunders and Moore (2013)
		M13Rx	CAGGAAACAGCTATGAC ACTTCTGG RTGICCRAARAAYCA	
	<i>Coccotylus</i>	GWSFn	TCAACAAAYCAYAAAGATATYGG3	Le Gall and Saunders (2010)
		GWSRx	ACTTCTGGRTGICCRAARAAYCA	Saunders and McDevit (2012b)
	<i>Devaleraea</i>	DevF1	CAGGAGTTATTGGAGGYTGT	Newly developed. Thermocycling regime followed Saunders and Moore (2013)
		M13Rx	CAGGAAACAGCTATGAC ACTTCTGG RTGICCRAARAAYCA	
rbcL-3P	Red algae	F57	GTAATTCCATATGCTAAAATGGG	Freshwater and Rueness (1994)
		rbcLrevNEW	ACATTTGCTGTTGGAGTYTC	Saunders and Moore (2013)
ITS	<i>Ahnfeltia</i>	P1	GGAAGGAGAAGTCGTAACAAGG	Saunders and Moore (2013)
		G4	CTTTTCCTCCGCTTATTGATATG	
	<i>Odonthalia</i>	P1	GGAAGGAGAAGTCGTAACAAGG	Newly developed. Thermocycling regime followed Saunders and Moore (2013)
		OdenG4	CTTTTCCTCCGCTTAATTATATG	

<i>Phycodrys</i>	P1	GGAAGGAGAAGTCGTAACAAGG	Newly developed. Thermocycling regime followed Saunders and Moore (2013)
	PhyG4	CTTTCCTGCGCTTAATAATATG	
<i>Rhodomela</i>	RLycF1	TAGGGGTACAGTGGTCTCAC	Newly developed. Thermocycling regime followed Saunders and Moore (2013)
	RLycR1	GAATCATTCGTCCTAAACGTC

1.040 Ma (0.386, 1.916)

* / 89
* / 86

0.77/*

0.79/60

Besa papillaeformis

Figure 1. Trans-Arctic genera of the orders Ahnfeltiales, Palmariales, and Gigartinales (Florideophyceae). Bayesian phylogenetic trees are presented for: A) *Ahnfeltia*; B) *Devaleraea*, *Halosaccion*, and *Palmaria*; C) *Coccotylus*, *Erythrodermis*, *Phyllophora*, and *Ozophora*; D) *Dilsea*, *Hyalosiphon*, and *Neodilsea*; E) *Mastocarpus*; and F) *Opuntiella* and *Turnerella*. Support values are presented at each node for posterior probabilities and Maximum-Likelihood bootstrap support, respectively. Nodes with greater than 0.95 posterior probability and/or 95% Maximum-Likelihood bootstrap support are indicated by an asterisk; nodes with less than 0.5 posterior probability and/or 50% bootstrap support are indicated with a negative symbol. *RbcL* saturation is indicated with a dashed line. Species genetically verified from the Arctic and/or the Northern Bering Sea are indicated with bold type.

Figure 2. Trans-Arctic genera of the order Ceramiales. Phylogenetic trees are presented for: A) *Melanothamnus*; B) *Membranoptera*; C) *Odonthalia*, *Neorhodomela*, and *Rhodomela*; D) *Polysiphonia*; E) *Phycodrys*; F) *Polyostea*; G) *Ptilota*; and H) *Vertebrata*. Support values are presented at each node for posterior probabilities and Maximum-Likelihood bootstrap support, respectively. Nodes with greater than 0.95 posterior probability and/or 95% bootstrap support are indicated by an asterisk; nodes with less than 0.5 posterior probability and/or 50% bootstrap support are indicated with a negative symbol. Species genetically verified from the Arctic and/or the Northern Bering Sea are indicated with bold type.

Figure 3. Divergence time estimates in species of Florideophyceae (Rhodophyta) that are hypothesized to have undergone trans-Arctic migration and subsequent speciation. The shaded area indicates the time frame for the Pleistocene (e.g. glaciation cycles present). The dashed line indicates the earliest hypothesized opening of the Bering Strait, while the square dot line indicates the latest hypothesized opening (Marincovich & Gladenkov, 1999). Error bars represent 95% minimum and maximum highest posterior density estimates. Species names refer to the lineage established from a trans-Arctic speciation event. Events in grey boxes should be interpreted as a single event (either the event occurred earlier or later in time).

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