Trans-Arctic vicariance in *Strongylocentrotus* sea urchins

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The sea urchins *Strongylocentotus pallidus* and *S. droebachiensis* first invaded the Atlantic Ocean from the Pacific following the opening of the Bering seaway in the late Miocene. While trans-Arctic dispersal during the Pleistocene is thought to have maintained species' integrity, a recent genomic analysis identified a reproductively isolated cryptic species within *S. droebachiensis*. Based on previous studies, the distribution of one of these lineages (*S. droebachiensis w*) includes the shallow water habitats of the northwest Atlantic and Pacific, while the other (*S. droebachiensis e*) is found throughout the shallow habitat in the northeast but is mostly restricted to deep habitats (>65m) in the northwest Atlantic. However, since genetic variation within *S. droebachiensis* has been largely unstudied in the north Pacific and Arctic oceans, the biogeography of the cryptic species is not well known, and it is difficult to identify the mechanisms driving population subdivision and speciation. Here we use population genetic analyses to characterize the distribution of each species, and to test hypotheses about the role of vicariance in the evolution of systematic and genomic divergence within the genus. We collected individuals of all 3 *Strongylocentrotus* species (n = 365) from 10 previously unsampled locations in the northeast Pacific and north Atlantic (Labrador Sea and Norway), and generated mtDNA sequence data for a 418 bp fragment of cytochrome *c* oxidase subunit I (*COI*). To assess the biogeography of all three species, we combined our alignment with 5 previously published data sets (*total n* = 789) and used statistical parsimony and maximum likelihood to identify species and characterize their distribution within and among oceans. Patterns of haplotype sharing, pairwise F_{ST}, and hierarchical analyses of molecular variance (AMOVA) identified trans-Arctic dispersal in *S. pallidus* and *S. droebachiensis w*, but other than 5 previously reported singletons we failed to detect additional mtDNA haplotypes of *S. droebachiensis e* in the north Pacific. Within the Atlantic, patterns of habitat segregation suggests that temperature may play a role in limiting the distribution of *S. droebachiensis e*, particularly throughout the warmer coastal waters along the coast of Nova Scotia. Our results are consistent with the cycles of trans-Arctic dispersal and vicariance in *S. pallidus*

and *S. droebachiensis w*, but we suggest that the evolution of Atlantic populations of *S. droebachiensis e* has been driven by persistent trans-Arctic vicariance that may date to the initial invasion in the late Pliocene.

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Abstract

The sea urchins *Strongylocentotus pallidus* and *S. droebachiensis* first invaded the Atlantic Ocean from the Pacific following the opening of the Bering seaway in the late Miocene. While trans-Arctic dispersal during the Pleistocene is thought to have maintained species' integrity, a recent genomic analysis identified a reproductively isolated cryptic species within *S. droebachiensis*. Based on previous studies, the distribution of one of these lineages (*S. droebachiensis w*) includes the shallow water habitats of the northwest Atlantic and Pacific, while the other (*S. droebachiensis e*) is found throughout the shallow habitat in the northeast but is mostly restricted to deep habitats (>65m) in the northwest Atlantic. However, since genetic variation within *S. droebachiensis* has been largely unstudied in the north Pacific and Arctic oceans, the biogeography of the cryptic species is not well known, and it is difficult to identify the mechanisms driving population subdivision and speciation. Here we use population genetic analyses to characterize the distribution of each species, and to test hypotheses about the role of vicariance in the evolution of systematic and genomic divergence within the genus. We collected individuals of all 3 *Strongylocentrotus* species (n = 365) from 10 previously unsampled locations in the northeast Pacific and north Atlantic (Labrador Sea and Norway), and generated mtDNA sequence data for a 418 bp fragment of cytochrome *c* oxidase subunit I (*COI*). To assess the biogeography of all three species, we combined our alignment with 5 previously published data sets (*total n* = 789) and used statistical parsimony and maximum likelihood to identify species and characterize their distribution within and among oceans. Patterns of haplotype sharing, pairwise F_{ST}, and hierarchical analyses of molecular variance (AMOVA) identified trans-Arctic dispersal in *S. pallidus* and *S. droebachiensis w*, but other than 5 previously reported singletons we failed to detect additional mtDNA haplotypes of *S.*

droebachiensis e in the north Pacific. Within the Atlantic, patterns of habitat segregation suggests that temperature may play a role in limiting the distribution of *S. droebachiensis e*, particularly throughout the warmer coastal waters along the coast of Nova Scotia. Our results are consistent with the cycles of trans-Arctic dispersal and vicariance in *S. pallidus* and *S. droebachiensis w*, but we suggest that the evolution of Atlantic populations of *S. droebachiensis e* has been driven by persistent trans-Arctic vicariance that may date to the initial invasion in the late Pliocene.

Introduction

The global biogeography of Strongylocentrotid sea urchins was shaped by the trans-Arctic interchange following the initial opening of the Bering seaway in the late Miocene (5.5-5.0 Mya; Marincovich & Gladenkov, 2001; Gladenkov et al., 2002), and fossil evidence reveals that *Strongylocentrotus droebachiensis* (along with many other Pacific taxa) reached western Europe by the late Pliocene (Durham & MacNeil, 1967). Following the initial invasion, eustatic sea level changes during the Pleistocene ice ages (2.4-0.2 Mya) periodically restricted dispersal across the Arctic Basin, causing widespread isolation and vicariance in the north Atlantic (Hewitt 1996; Cunningham & Collins, 1998). As a result of these processes, molecular evidence from trans-Arctic taxa indicates a complex pattern of inter- and intra-specific divergence, with species positioned along a continuum between complete reproductive isolation and panmictic populations (see Laakkonen et al., 2021). There has been consensus that both genetic diversity and species integrity have been maintained among populations of *Strongylocentrotus* sea urchins from the Pacific (ancestral) and Atlantic (colonized) as a result of gene flow across the Arctic between 0.40-0.11 Mya (Palumbi & Kessing, 1990; Palumbi & Wilson, 1991; Addison & Hart,

2005; Laakkonen et al., 2021). However, the recent discovery of a cryptic species within the north Atlantic population of *S. droebachiensis* (Addison & Kim, 2018) suggests a more complicated role of vicariance in the evolution of the genus, demanding a re-evaluation of both the biogeography and population genetics throughout the range.

Like many marine invertebrates, *Strongylocentrotus* sea urchins are broadcast spawners with long-lived planktonic larvae (4 to 21 weeks; Strathmann, 1978) capable of high rates of dispersal with gene flow. Genetic studies of *S. droebachiensis* (Müller, 1776) in the north Atlantic detected local panmixis in the northwest (Addison & Hart, 2004, 2005), a small but significant latitudinal gradient in the northeast (Nordberg et al., 2016), and significant population substructure between the east and west coasts (Addison & Hart, 2004, 2005; Harper et al., 2007). Patterns of genetic variation at both microsatellites and mtDNA reveal lower levels of diversity in the eastern populations compared to the west, and pairwise F_{ST} suggests that northwest populations are genetically more similar to the Pacific than to those from the northeast Atlantic (Addison & Hart, 2004, 2005). A more detailed study of *S. droebachiensis* in the northwest Atlantic indicates strong habitat segregation and reproductive isolation between distinct east and west mtDNA haplogroups, where the vast majority shallow-water coastal samples (<30m) were a subset of haplotypes shared between the Pacific and the northwest Atlantic, and the offshore deep-water (>65m) samples were identical to (or clustered with) populations from Norway and Iceland (Addison & Kim, 2018). These genetic patterns broadly correspond to variation in sperm morphology reported between sea urchins from the northeast Atlantic and those from the northwest Atlantic and Pacific (Manier & Palumbi, 2008; Marks et al., 2008). Together, these patterns of genetic and morphological divergence indicate that trans-Atlantic variation reflects

81 species level differences and are not the result of limited gene flow and genetic drift between
82 allopatric populations. Thus, there is strong evidence that the north Atlantic harbours two
83 reproductively isolated species of *Strongylocentrotus* sea urchins: *S. droebachiensis w* whose
84 distribution includes the shallow water habitats of the northwest Atlantic and Pacific, and *S.*
85 *droebachiensis e* that is distributed throughout the shallow habitat in the northeast and deep
86 habitats (>65m) at lower latitudes in the northwest Atlantic.
87
88 Cycles of trans-Arctic dispersal and vicariance have played an important role in the evolution of
89 new species in a variety of Pacific and Atlantic taxa, including molluscs, crustaceans,
90 echinoderms, polychaetes, fishes, mammals, and algae (e.g., Wares 2001; Carr et al., 2011;
91 Layton et al., 2016; Neiva et al., 2018; Bringloe et al., 2020; Laakkonen et al., 2021). The
92 surprising discovery of two independent lineages of *S. droebachiensis* suggests that allopatric
93 speciation in this genus may have also followed the initial trans-Arctic invasion. While
94 vicariance during the Pliocene-Early Pleistocene resulted in the speciation of the sea stars
95 *Asterias forbesi* and *A. rubens* in the Atlantic (Wares, 2001), more recent vicariant histories
96 during the Middle Pleistocene (1-0.2 Mya) have resulted in reciprocal monophyly and
97 divergence among populations of *Solaster endeca, Pteraster militanus*, and *Crosster papposus*
98 (Layton et al., 2016). Interoceanic divergence between allopatric Pacific and Atlantic lineages of
99 these species ranges from 1.24% to 2.98%, and although the taxonomic status in these groups is
100 unknown, these differences are comparable to species level differences at cytochrome *c* oxidase
101 subunit I sequences (*COI*) among other echinoids (Echinometra 2-3%, Palumbi et al., 1997;
102 Leptasterias 0.4-2.2%, Hrincevich et al., 2000; Patiriella 1.1-4.3%, Hart et al., 2003), including
103 the cryptic lineages of *S. droebachiensis* (2.3%, Addison & Kim, 2018). However, the

104 distribution and extent of the ecological segregation of both lineages of *S. droebachiensis*
105 throughout the north Atlantic are not well known, particularly in the Labrador Sea, where the
106 west flowing Greenland Current is expected to connect populations across the north Atlantic
107 (Knutsen et al., 2007; Bringloe et al., 2020). Furthermore, genetic variation within *S.*
108 *droebacheisis* has been largely unsampled in the north Pacific, making it difficult to assess the
109 role of trans-Arctic vicariance to patterns of evolution within the genus.
110
111 Here we extend analyses of biogeography and population genetic structure in circumpolar
112 *Stronglyocentrotus* sea urchins to better understand the roles that trans-Arctic and trans-Atlantic
113 dispersal have played in the systematic and genetic divergence within the genus. We aim to
114 establish a more complete understanding of the range limits of each species by compiling
115 previous surveys of mtDNA sequence variation and including additional sample sites for *S.*
116 *pallidus* and *S. droebachiensis* throughout the Pacific and north Atlantic. *Strongylocentrotus*
117 *pallidus* is a circumpolar species that is abundant in shallow water (<15 m) in the north, and
118 deeper waters of up to 1600 m at lower latitudes (Jensen, 1974; Strathmann, 1981; Gagnon &
119 Gilkinson 1994; Bluhm et al., 1998). The current known distribution of *S. droebachiensis w*
120 includes the northeast Pacific and the shallow water habitat of the northwest Atlantic, and *S.*
121 *droebachiensis e* is the only green sea urchin found in the northeast Atlantic and in the deep
122 offshore habitat in the northwest Atlantic. However, there is less certainty about the full
123 distribution *of S. droebachiensis e*, because although it appears to be circumpolar, it was only
124 detected at low frequency in the Pacific (5/29 samples; Addison & Hart, 2005), and none of these
125 haplotypes were shared with the Atlantic suggesting a lack of recent trans-Arctic dispersal.
126 Although mtDNA from this lineage was extremely rare at lower latitudes in the shallow

northwest Atlantic habitat (6/161 samples; Addison & Hart, 2005; Addison & Kim, 2018), a more complete understanding of the biogeography within the genus requires additional sampling at higher latitudes in both the northwest Atlantic and northeast Pacific. Late glacial and post-glacial trans-Arctic dispersal between populations of *S. pallidus* and *S. droebachiensis w* has resulted in both shallow inter-ocean divergence and widespread sharing of haplotypes (Palumbi & Kessing, 1990; Palumbi & Wilson, 1991; Addison & Hart, 2005; Laakkonen et al., 2021). Since the coastal habitat at high latitudes in the Pacific and northwest Atlantic is qualitatively similar to the northeast Atlantic where *S. droebachiensis e* dominates (e.g., cool water, kelp; Payne et al., 2012; Government of Canada, 2014; Bringloe & Saunders, 2019), we expect concordant biogeography and patterns of genetic diversity in *S. droebachiensis e* if it shared a similar history of invasion, vicariance, and secondary contact. However, an absence of haplotype sharing or discordant biogeography would suggest a lack of trans-Arctic dispersal (persistent vicariance), raising the possibility that *S. droebachiensis e* diverged in allopatry following the initial trans-Arctic invasion in the late Pliocene. This study will provide insight into the mechanisms driving reproductive isolation in the northern lineages of *Strongylocentrotus* sea urchins by defining the biogeographical distribution of allopatric and sympatric populations, and quantifying genetic subdivision both within and between oceans.

Materials & Methods

Sampling, DNA extraction, amplification, and sequencing

To examine the range distributions of all three lineages of *Strongylocentrotus* sea urchins we compiled *COI* sequence data from previous studies (GenBank accession Nos. AY504479-AY504511, Addison & Hart, 2005; EF108346-EF108365, Harper et al., 2007; MG098337-

MG098440, Addison & Kim, 2018; MT736172-MT736220, Laakkonen et al., 2021), and collected new samples from 10 locations throughout the north Atlantic and northeast Pacific oceans (Fig. 1; Table 1). New samples were collected from most sites at depths of 2-8 meters using SCUBA, except in the Bay of Fundy (14-90m) where samples were collected using a fixed gear dredge, and Owl's Head Nova Scotia (60m) where collections were made using baited lobster traps as described in Filbee-Dexter & Scheibling (2014). Sea urchins were collected with permission under Section 52 permits (Department of Fisheries and Oceans Canada: 356132, NL-2619-14, TNMP-2014-16478, and S-14/15-1053-NU), and the State of Alaska Department of Fish and Game (CF-17-004). Sea urchins from the northeast Atlantic at Kongsfjord and OsloFijord (Norway) were a subset of those analysed by Norderhaug et al. (2016) for which we generated new mtDNA sequence data. We generally observed the colour characteristics of the specimens (i.e., test, tube feet, and aboral spines) described in Jensen (1974) and Gagnon & Gilkinson (1994), but since the structures used to distinguish the species are challenging to observe under field conditions (e.g., pedicellariae, spine wedges, and pore pairs), designations were ultimately made using DNA sequence data (*see* Addison & Kim, 2018). We preserved gonad tissue and/or tube feet in 95% ethanol, and extracted total genomic DNA using DNAeasy Blood and Tissue columns (QIAGEN) following the manufacturer's recommended protocols.

We targeted a fragment of the cytochrome oxidase subunit I (*COI*) mitochondrial gene using the polymerase chain reaction (PCR) primers COIJ and COIC (Edmands et al., 1996). Following Addison and Kim (2018), we performed amplifications in a 30µL volume consisting of ~4ng DNA, 1× ThermoPol reaction buffer (New England Biolabs, NEB), 0.2mmol dNTPs (NEB), 2.0mmol $MgSO_4$, 0.5µmol forward and reverse primers, and 1.0 unit of Taq polymerase (NEB).

Thermal cycling conditions were 95°C for 3min, followed by 39 cycles of 95°C (30s), 45°C (30s), 72°C (60s), and a final extension at 72°C for 3min. We checked amplicons using agarose gel electrophoresis and visualized with SYBR™ Safe (Invitrogen™) under UV light. Sanger sequencing using forward, reverse, or both PCR primers was conducted at the Genome Quebec Innovation Centre (McGill University, Montreal, Quebec, Canada). Sequences were edited, aligned, and trimmed to a length of 418bp in SEQUENCHER, version 5.0 (Gene Codes; GenBank accession No. OL451446 – OL451529, OL451534 – OL451866).

Polymorphism and population genetic structure

We identified individuals as *Strongylocentrotus pallidus*, or one of the two reproductively isolated cryptic lineages of *S. droebachiensis* (*see* Addison & Kim, 2018) using a combination of maximum likelihood and statistical parsimony. We inferred a phylogenetic tree of unique haplotypes by maximum likelihood using PHYML 3.0 (Guindon et al., 2010), with an HKY85 substitution model, gamma distributed rate heterogeneity at sites, and an SPR tree search. Node support for the putative species clusters was estimated using nonparametric bootstrap analysis with 1000 replicates. To visualize species assignment, we used statistical parsimony implemented in TCS v.1.21 (Clement et al., 2000) and presented using PopART (http://popart.otago.ac.nz). Mean genetic distances (K2P, Kimura two-parameter distances; Kimura, 1980) within and between lineages were calculated in MEGA (Kumar et al., 2018; Stecher et al., 2020). We calculated genetic diversity for each species following Addison and Kim (2018). Measurements included: nucleotide diversity (π), number of segregating sites (S), number of haplotypes (H), and haplotype diversity (h) for each sampling location using DNASP v.5.1 (Librado & Rozas, 2009). We tested for departures from neutrality based on allelic states or

196 segregating sites with Fu's F_S (Fu, 1997) and Tajima's D (Tajima, 1989), respectively, using
197 ARLEQUIN (Excoffier & Lischer, 2010). For neutral or near-neutral evolving markers such as
198 mtDNA, significantly negative values of these tests can indicate a higher-than-expected number
199 of single mutations (D) or haplotypes (F_S) which can result from population expansion (Ramos-
200 Onsins & Rozas, 2002). While both tests are frequently used to distinguish between models of
201 population growth or no-growth, simulations have observed that Fu's F_S has greater power to
202 detect population growth (Ramos-Onsins & Rozas, 2002). Significance was assessed by 10,000
203 coalescent simulations. To control for the occurrence of false positives due to multiple
204 comparisons, significance of the p-values was determined using the Bonferroni correction. To
205 simultaneously visualize both the phylogenetic relationships and the frequency of each
206 haplotype, we constructed separate haplotype networks for each species using statistical
207 parsimony implemented in TCS v.1.21 (Clement et al., 2000) and presented using PopART.
208
209 To evaluate the genetic subdivision among populations of each lineage within and between
210 major oceanographic regions, we calculated global F_{ST} and tested for pairwise genetic
211 differences between populations. We conducted analyses of molecular variation (AMOVA) to
212 test for hierarchical genetic structure both within and among the Pacific and Atlantic Oceans
213 (Fig. 1). We also explored *post hoc* hypotheses based on patterns of pairwise F_{ST} to further
214 refine patterns of substructure. Indices of genetic differentiation (F_{ST} and Φ) were calculated
215 using Kimura two-parameter distances (K2P: Kimura, 1980) implemented in ARLEQUIN, and
216 significance was assessed using 10,000 permutations of the data with Bonferroni correction for
217 multiple tests.
218

Results

We obtained 418 bp *COI* sequences (positions 6415–6832 of Jacobs et al., 1988) for 789 individual sea urchins. There were 60 variable sites and a total of 83 unique haplotypes. Based on maximum likelihood and statistical parsimony analyses (Fig. 1c) our results support the presence of three lineages reported in Addison & Kim (2018). We detected three distinct clusters of haplotypes including *S. pallidus* (n = 156), and both lineages of *S. droebachiensis* (*S. droebachiensis e*, n = 148; *S. droebachiensis w*, n = 485) (Table 2). Mean genetic distance among all the sequences (K2P) was 2.40%, while within lineage mean genetic distance ranged from 0.33% to 0.41% (Fig. 1). Pairwise genetic distance was the greatest between *S. pallidus* and *S. drobachiensis w*, while the cryptic lineages within *S. droebachiensis* were 2.73% divergent. For all 3 lineages, the net between group distances (K2P) were 6-28x greater when comparing samples between oceans (i.e., trans-Arctic) than between the east and west coasts of the same ocean (trans-Pacific or trans-Atlantic; Table 3). Overall, haplotype (*h*) and nucleotide (π) diversity was high in all three lineages, and values ranged from 0.685-0.733 and 0.033-0.0040, respectively (Table 2). Significant negative values of Fu's F_S (-6.600 to -4.400) suggest a demographic expansion (or purifying selection) in samples of *S. pallidus* and *S. droebachiensis e* from offshore sites (>60m) in the northwest Atlantic, and *S. droebachiensis w* from OKH and NOM in northeast Pacific (Table 2). Sea urchin populations of all three lineages did not show an excess of alleles in shallow water habitats throughout the northwest Atlantic.

Biogeography

Strongylocentrotus pallidus was distributed in high relative abundance across all three oceanographic regions sampled. Pure populations of *S. pallidus* were detected above the Arctic

Circle in both the northwest and northeast Atlantic (Fig. 1), and this species was relatively abundant (13-25%) in mixed aggregations at shallow sites along the coast of Labrador and western Newfoundland. However, *S. pallidus* was rare at all other shallow water sites in Atlantic Canada (6 of 236 total samples; 2.5%), including 1 individual at 14m in the Bay of Fundy where it was absent at depths >70m. In contrast, *S. pallidus* was common at deeper sites, making up 28% of the samples collected at 60 m off the coast at Owl's Head, NS (19 of 69), and 69.6% of the samples collected Offshore at depths >65m on the Scotian Shelf (39 of 56).

The green sea urchin, *S. droebachiensis*, was detected across all three oceanic regions sampled, but there were striking differences in the distribution of the cryptic lineages. *Strongylocentrotus droebachiensis e* was the only green sea urchin found in the northeast Atlantic, where pure populations were sampled throughout Iceland, Norway, and Russia (Fig. 1). In the northwest Atlantic, *S. droebachiensis e* shared a distribution similar to *S. pallidus* where it made up ~12% of the samples at shallow sites at higher latitudes throughout the Labrador Sea and the Gulf of St Lawrence (20/162). However, this lineage was rare in the shallow coastal samples (<60 m) at lower latitudes, comprising only ~1% of the individuals sampled along Nova Scotia and the Bay of Fundy (2 of 201), and was the only green sea urchin found offshore on the Scotian Shelf. With the exception of the 5 individuals from the San Juan Island site reported in Addison & Hart (2005), we failed to detect additional samples of *S. droebachiensis e* throughout the north Pacific.

Strongylocentrotus droebachiensis w was distributed throughout the Pacific and coastal samples from Atlantic Canada. With the exception of the low frequency and geographically isolated

haplotypes of *S. droebachiensis e* detected at SJI, all other green sea urchin samples collected throughout the Pacific Ocean were identified as *S. droebachiensis w* (Fig. 1). In the northwest Atlantic, *S. droebachiensis w* was detected at all coastal sites (including OWH and BOF) where it comprised an increased proportion of the samples at lower latitudes (Fig. 1). With the exception of the pure population of *S. pallidus* sampled at QIK, samples of sea urchins collected in the Gulf of St. Lawrence and the Labrador Sea consisted of 73.4% *S. droebachiensis w* (116 of 157) compared to 86.6% (258/298) of those sampled along Nova Scotia and the Bay of Fundy at the southern end of its' range. When the deep-water samples in the Bay of Fundy (70-90m) and Owl's Head (60 m) are removed, the proportion of the *S. droebachiensis w* lineage in the shallow habitat (<30 m) throughout Nova Scotia and the Bay of Fundy increases to 95% (191 of 201).

Population genetic structure

Strongylocentrotus pallidus

Range-wide genetic structure in *S. pallidus* was primarily driven by differences between the Pacific and Atlantic samples, and an absence of genetic subdivision within the north Atlantic. Statistical parsimony identified a single abundant and geographically widespread genetic variant (50% of all samples) distributed across all three major oceanographic regions. In addition to this shared haplotype, there was some inter-ocean divergence as half of the haplotypes detected in both the Pacific and the shallow water northwest Atlantic were exclusive to those regions (Fig. 2). In contrast, with only two exceptions, all the *S. pallidus* samples from Norway (PSF) shared a single *COI* haplotype. Multi-locus nuclear genotypes have been scored for 9 of the 20 individuals (all from ACS) harbouring the three unique northwest Atlantic *S. pallidus* haplotypes (Fig. 2), all of which have been identified as *S. droebachiensis w* (Addison & Hart, 2005;

288 Addison & Kim, 2018; Burke, Kim, & Addison *in prep*) suggesting historic hybridization and
289 introgression (Addison & Pogson, 2009). Since these haplotypes have been segregating in *S.*
290 *droebachiensis w* for many generations, they do not reflect the biology of *S. pallidus* and thus we
291 removed them from subsequent analyses of population genetic structure. Global F_{ST} was high
292 (0.2816; $P < 0.001$), indicating strong and significant variance in the distribution of genetic
293 variation. Pairwise F_{ST} values were high between the Pacific (SJI) and the northwest Atlantic
294 samples from (OWH and ACO) and northeast Atlantic (PSF; Pairwise F_{ST} = 0.2998-0.3704, $P <$
295 0.003; Table 4), suggesting limited dispersal with gene flow across the Arctic basin. In contrast,
296 there was not a significant difference between sample sites within the north Atlantic (Pairwise
297 F_{ST} = 0-0.0689, $P > 0.003$). Hierarchical analysis of molecular variance (AMOVA) based on the
298 *a priori* grouping of populations from each oceanographic region were not significant (Φ_{CT} =
299 0.353; $P = 0.205$), and we failed to detect evidence of sub-structure based on geography or depth
300 within the Atlantic (Table 5).
301
302 *Strongylocentrotus droebachiensis e*
303 Patterns of genetic subdivision were largely driven by differences between the Pacific and
304 Atlantic basins, and low substructure within Atlantic. We detected 7 *COI* haplotypes *S.*
305 *droebachiensis e* sampled from Norway and Iceland, four of which were also widespread and
306 abundant in the northwest Atlantic (Fig. 3). Two of the three unique haplotypes in the northeast
307 Atlantic were detected in the easternmost Arctic sites in Norway (VAR) and Russia (KOP).
308 While *S. droebachiensis e* was detected throughout the northwest Atlantic, the majority of the
309 individuals were found at deep sites (>60m) and had rare mtDNA haplotypes. Of the 28 unique
310 haplotypes detected for this lineage, 21 were only detected once, and of those 14 were found in

311 the northwest Atlantic. None of the 5 *COI* haplotypes genotyped at SJI in the Pacific were found
312 in the Atlantic populations. Global F_{ST} was lower for this lineage compared to the others (F_{ST} =
313 0.2052; P <0.001), indicating a moderate level of genetic subdivision throughout the range.
314 Pairwise comparisons revealed strong divergence between both SJI (Pacific), and ND2 (Norway)
315 from most other sites (Table 6). Genetic subdivision was generally low and not significantly
316 different from zero among most locations across the Atlantic Basin. Hierarchical AMOVA
317 indicated strong regional grouping based on the oceanographic regions within the northeast
318 Atlantic (Φ_{CT} = 0.3287, P < 0.0001; Table 5, AMOVA), but we failed to detect significant
319 variation across the north Atlantic or among the *a priori* grouping of sampling sites across
320 oceanographic basins.
321
322 *Strongylocentrotus droebachiensis w*
323 Genetic variation within *S. droebachiensis w* was consistent with previous studies, with strong
324 differences across the Arctic and genetic homogeneity among southern coastal sites in the
325 northwest Atlantic. However, shared halpotypes and generally lower pairwise F_{ST} values (Table
326 7) among new samples from the Labrador Sea and north Pacific suggest a greater influence of
327 trans-Arctic dispersal. Unique haplotypes were found in both the Pacific and northwest Atlantic,
328 but three high frequency genetic variants were shared throughout both oceans (Fig. 4). Based on
329 both the distribution of haplotypes and pairwise F_{ST} among new samples, sites from the Labrador
330 Sea were generally more similar to sites in the north Pacific than to the northwest Atlantic.
331 Global F_{ST} was high (0.4337; P<0.0001), and was largely driven by the differences between the
332 southern samples of the northwest Atlantic (i.e., the Gulf of St. Lawrence, Nova Scotia, and the
333 Bay of Fundy) and those from the Labrador Sea and the north Pacific (Table 7). Consistent with

334 earlier studies (Addison & Hart, 2004; 2005) sites throughout the Gulf of St. Lawrence, Nova
335 Scotia, and the Bay of Fundy were genetically homogeneous (pairwise F_{ST} = 0 to 0.0400, $P >$
336 0.10). There was a striking correlation between latitude and genetic similarity among the
337 northern sites in the Pacific and the Labrador Sea. Pairwise F_{ST} was not significant between
338 NOM (Alaska; 64.487°N) and DUR (Nunavut; 67.038°N), but these sites were different from the
339 next closest sample in each region (Table 7). A similar pattern in the magnitude of the pairwise
340 F_{ST} was observed between OKH (Russia; 59.494°N), QCI (British Columbia; 54.193°N) and
341 both NAI (Labrador; 56.504°N) and MAK (Labrador; 55.102°N). This latitudinal pattern was
342 driven by differences in the identity of the single most abundant haplotype at sample sites within
343 each group. The most frequent haplotype at OKH, QCI, NAI, and MAK (0.69, 0.77, 0.74, and
344 0.70, respectively), was the second most frequent haplotype at NOM (0.23) and DUR (0.28).
345 Although genetic structure based on the *a priori* grouping of samples in the Pacific and Atlantic
346 was significant (Φ_{CT} = 0.3184; P = 0.0349; Table 5), as was our grouping of samples by latitude
347 (Φ_{CT} = 0.3955; P = 0.0146), exploration of the results maximized the resolution of geographic
348 subdivision when we included four distinct groups of sea urchins in the east and west Pacific,
349 Labrador Sea, and coastal northwest Atlantic (Φ_{CT} = 0.49691; P = 0.0013).
350
351

352 ## Discussion

353 The biogeographic distribution, concordant population genetic structure, and patterns of
354 haplotype sharing among oceanic regions suggest that cycles of vicariance and trans-Arctic gene
355 flow has shaped diversification within circumpolar Strongylocentrotid sea urchins. While there
356 is considerable debate about the competing contributions of both geographic isolation and

357 divergence with gene flow to the process of speciation in the sea (e.g., Miglietta et al., 2011;
358 Faria et al., 2021), our results suggest that isolation across the Arctic Basin has been a driving
359 force of genomic and systematic diversity within the genus. Consistent with earlier studies
360 (Palumbi & Wilson, 1990; Palumbi & Kessing, 1991; Addison & Hart, 2004; 2005; Harper et al.,
361 2007), we detected widespread sharing of identical haplotypes throughout the Pacific and
362 Atlantic populations of *S. pallidus* and *S. droebachiensis w*, and patterns of population genetic
363 subdivision among these regions suggests recent interoceanic exchange. However, there was no
364 evidence of a similar pattern of trans-Arctic dispersal in *S. droebachiensis e*, as we failed to
365 detect haplotypes from this species at additional sample sites throughout the north Pacific.
366 Following the initial trans-Arctic invasion of the north Atlantic by Pacific ancestors, our results
367 suggest that *Strongylocentrotus droebachiensis* diverged into reproductively isolated cryptic
368 species, one of which remains connected with the pacific (*S. droebachiensis w*) while the other is
369 now endemic to the Atlantic (*S. droebachiensis e*). Although the strong patterns of hierarchical
370 population structure within *S. droebachiensis w* suggests a contribution of latitude to the
371 distribution of genetic variation, our analysis of a putatively neutral mtDNA locus does not
372 display a similar signature of adaptive evolution in response to temperature driven by latitudinal
373 variation reported for populations of Atlantic cod (Bradbury et al., 2010) and Atlantic salmon
374 (Jeffery et al., 2017). Our results suggest that repeated trans-Arctic gene exchange contributed to
375 the maintenance of species integrity in two species, while vicariance may have contributed to the
376 allopatric speciation of a third species in the north Atlantic.
377
378 Like many arctic-boreal marine species, *Strongylocentrotus* sea urchins with a circumarctic
379 distribution have experienced cycles of isolation and invasion throughout the Pleistocene ice

ages (e.g., Laakkonen et al., 2021). Following the trans-Arctic invasion in the late Pliocene, allopatric populations of Pacific and Atlantic sea urchins diverged throughout the Pleistocene when cycles of glacial advance and retreat (ca 2.4-3.0 Mya) restricted dispersal around the Bering Strait (Einarsson et al., 1967; Herman & Hopkins, 1980; Maslin et al., 1996; Haug et al., 1999; Harris 2005; Lisiecki & Raymo, 2005; Horikawa et al., 2015; Lozea-Quintana et al., 2019). The presence of multiple private haplotypes in Nova Scotia and New Brunswick suggests that *S. droebachiensis w* persisted in refugia at the southern end of their northwest Atlantic range, but moderate genetic structure and sequence similarity among oceans indicates that Pacific populations subsequently re-invaded the Atlantic during interglacial periods throughout the Pleistocene (Hewitt, 2003; Maggs et al., 2008). We detected patterns of genetic subdivision and sequence diversity within *S. pallidus* that are consistent with *S. droebachiensis w*, although the small sample size of the hierarchical analysis (3 groups, 6 sites) suffered from low power and could not approach significance at the 5% threshold (Fitzpatrick, 2009). Genetic analysis of new samples collected at high latitudes in both oceans revealed extensive haplotype sharing and lower pairwise F_{ST} values compared to studies conducted at more southern latitudes (Palumbi & Wilson, 1990; Palumbi & Kessing, 1991; Addison & Hart, 2004; 2005; Harper et al., 2007). These results indicate that trans-Arctic connectivity is likely greater than previously reported, and while qualitatively consistent with coalescent analyses (Laakkonen et al., 2021) that show the predominant migration vectors track the east flowing currents connecting the Pacific with the northwest Atlantic (Ledu et al., 2008), we cannot exclude a hypothesis of back migration from the northwest Atlantic to the Pacific (*see* Addison & Hart, 2005; Harper et al., 2007). While Pacific and northwest Atlantic populations of *S. pallidus* and *S. droebachiensis w* experienced

402 periods of vicariance throughout the Pleistocene, late glacial and post-glacial trans-Arctic
403 dispersal continues to maintain the integrity of these species.
404
405 Patterns of biogeography and genetic diversity suggest that *S. droebachiensis e* is almost
406 exclusively limited to the Arctic and sub-Arctic in north Atlantic. Additional sampling
407 confirmed that this lineage is the only green sea urchin found in the northeast Atlantic, and that
408 its range in the northwest Atlantic is characterized by a clear shift to deeper habitats at lower
409 latitudes. While *S. droebachiensis e* was present at some shallow sites in the Canadian Arctic,
410 Labrador Sea, and Gulf of St. Lawrence, our sampling efforts at similar latitudes in the Pacific
411 failed to detect additional evidence of this species beyond those reported by Addison & Hart
412 (2005). Since the sites at Haida Gwaii (LAN, MAS), Sea of Okhotsk (OKH), and the Bering Sea
413 (NOM) share similar coastal temperatures and macroalgal assemblages (Payne et al., 2012;
414 Government of Canada 2014; Bringloe & Saunders, 2019) as those supporting *S. droebachiensis*
415 *e* in the north Atlantic, failure to detect additional representatives suggests they are not broadly
416 distributed throughout the Pacific. In addition, the 5 singleton haplotypes of *S. droebachiensis e*
417 reported from the San Juan Islands (Addison & Hart, 2005) were not identified in the north
418 Atlantic, indicating very limited (or complete absence) of trans-Arctic gene flow in this species.
419 These results suggest that the presence of *S. droebachiensis e* haplotypes in the Pacific could
420 represent incomplete lineage sorting of ancestral alleles, or possibly low levels of back migration
421 of *S. droebachiensis e* individuals or haplotypes (via introgression into *S. pallidus* or *S.*
422 *droebachiensis w*; Addison & Hart, 2005; Harper et al., 2007) during interglacial periods
423 throughout the Pleistocene. While additional analyses of both coastal and deep habitats
424 throughout the Pacific are required before concluding that *S. droebachiensis e* is absent from the

Pacific Ocean, our findings suggest this species may have evolved in allopatry following the initial trans-Arctic invasion of *S, droebachiensis* during the late Pliocene. Alternatively, the two lineages of *S. droebachiensis* could have initially diverged in the Pacific prior to invading the Atlantic, followed by a subsequent reduction (or possibly extirpation) *S. droebachiensis e* in the Pacific. At the very least, our study reveals that, unlike *S. pallidus* and *S. droebachiensis w*, Pacific and Atlantic populations of *S. droebachiensis e* continue to diverge in a state of persistent trans-Arctic vicariance.

The repeated trans-Arctic dispersal of *S. pallidus* and *S. droebachiensis w* following the initial period of vicariance suggests that the northwest Atlantic is a zone of secondary contact between all three species. Early studies employing microsatellites (Addison & Hart, 2005), nuclear DNA sequences (Addison & Pogson, 2009), and single nucleotide polymorphisms (SNPs; Addison & Kim, 2018) detected mitochondrial and nuclear discordance in 9 of the 305 (3.0%) individual sea urchins analyzed throughout the Pacific and Atlantic oceans. In these studies, all the hybrid individuals identified were a result of introgression of *S. pallidus* mtDNA into *S. droebachiensis* individuals. For example, Addison & Kim (2018) tested for evidence of hybridization using both *COI* sequences and 3,049 nuclear SNPs in a sample of 110 sea urchins collected along a depth gradient off the coast of Nova Scotia. While 2 *S. droebachiensis w* individuals from shallow sample sites harboured *S. pallidus* mtDNA, the lack of admixture across the nuclear genome of all samples provides evidence against widespread contemporary hybridization and suggests that reproductive isolation is complete. In Addition, patterns of endemism of the introgressed haplotypes in both oceans suggests that historic introgressive hybridization from *S. pallidus* into *S. droebachiensis w* may have occurred independently in Pacific and Atlantic populations.

448 Previous studies have not revealed evidence of hybridization between *S. droebachiensis e* and
449 the other two species. However, extensive analyses of both nuclear and mitochondrial DNA
450 throughout the northwest Atlantic are needed to test the hypothesis that contemporary hybrids
451 form under natural spawning conditions, particularly at sites where all 3 species co-occur (e.g.,
452 OWH and NAI).
453
454 While trans-Arctic vicariance is the dominant mechanism driving the initial divergence of *S.*
455 *droebachiensis e* from ancestors in the Pacific, allopatry within the Atlantic has contributed to
456 patterns of divergence in other echinoderms. Beginning in the mid-Pliocene, rapid ocean cooling
457 and the formation of the Labrador current isolated temperate north Atlantic species where
458 warmer mid-Atlantic and Gulf stream waters provided refuge on north American and European
459 coasts (Berggren & Hollister, 1974; Worley & Franz, 1983; Cronin, 1988; Wares, 2001).
460 Genetic evidence supports this hypothesis in sea stars, where western *Asterias forbesi* and
461 eastern *A. rubens* diverged in allopatry followed by the post-glacial recolonization and sympatry
462 in the northwest Atlantic (Wares, 2001; Wares & Cunningham, 2001). These species now form
463 a secondary contact zone from Nova Scotia to Cape Cod, and laboratory studies of sperm
464 competition (Harper & Hart, 2005), morphology, and genetic surveys of natural populations
465 (Harper & Hart, 2007) have identified hybridization and introgression. While patterns of
466 ecological, morphological, and genetic divergence identified within *S. droebachiensis* are
467 qualitatively similar to those for *Asterias*, our results only weakly fit the scenario of post
468 Pliocene divergence of allopatric populations within the north Atlantic. Support for this
469 hypothesis includes evidence of reproductive isolation between the east and west lineages
470 (Addison & Kim, 2018), habitat segregation of the eastern lineage in the west, and a signal of

471 range expansion in western samples of *S. droebachiensis e* and the co-distributed population of
472 *S. pallidus*. However, we failed to detect moderate or weak population genetic structure typical
473 of recent trans-Atlantic dispersal (Young et al., 2002; Provan et al., 2005; Chevolot et al., 2006;
474 Jolly et al., 2006; Hoarau et al., 2007; Souche et al., 2015; Andrews et al., 2019; Neiva et al.,
475 2020), and in contrast, we identified more private haplotypes and higher genetic diversity (h, π)
476 in northwest Atlantic samples of both *S. pallidus* and *S. droebachiensis e*. These patterns suggest
477 that sea urchins in the northwest Atlantic have persisted in single or multiple glacial refugia
478 (Hewitt, 2003; Maggs et al., 2008), and were unlikely to have been extirpated during glacial
479 maxima throughout the Pleistocene. Although repeated cycles of isolation and dispersal between
480 the east and west coasts throughout the Pleistocene may have obscured signals of historic
481 vicariance (Jesus et al., 2006; Maggs et al., 2008), our results suggest that lineages of *S.*
482 *droebachiensis* have not been strictly allopatric within the north Atlantic following the initial
483 invasion, and that vicariance within the Atlantic was not the principal driver of speciation within
484 the genus.
485
486 Identifying the mechanisms driving speciation in the sea can be challenging because of the
487 difficulty in identifying barriers to gene exchange, or the environmental factors driving
488 adaptation. Addison & Kim (2018) suggest that tolerance of seasonally lower salinity may
489 contribute to the ecological segregation of the *Strongylocentrotus* lineages in the southern part of
490 their western Atlantic range (e.g., along the coast of Nova Scotia). In this study, we identified
491 contrasting patterns of geographic distribution and habitat segregation that suggest increased
492 water temperatures in the northwest may contribute to the near complete absence of *S.*
493 *droebachiensis e* from shallow sites dominated by *S. droebachiensis w.* In the northwest

Atlantic, larvae of *S. droebachiensis* grow rapidly at 14°C (Hart & Scheibling, 1988), and in the Pacific and northwest Atlantic both larvae and adults can withstand temperatures up to 19.7 and 22°C, respectively (Scheibling & Stephenson, 1984; Pearce et al., 2005). Like other species with planktonic dispersing larvae, *S. droebachiensis* exhibits large regional and interannual fluctuations in recruitment (e.g. Raymond & Scheibling, 1987; Scheibling & Raymond, 1990; Scheibling, 1996), but is known to settle along the coast of Nova Scotia in July when water temperature can exceed 14°C (Balch & Scheibling, 2000). In August and September, the nearshore water temperatures along the coast of Nova Scotia regularly reach 20°C (Scheibling et al., 2013). In contrast, water temperatures along the Norwegian coast are comparatively cooler (Danielssen et al., 1996; Ibrahim et al., 2014), and green sea urchins experience recruitment failure in kelp beds at southern latitudes when temperatures exceed 10°C (Fagerli et al., 2013; Rinde et al., 2014; Nyhagen et al., 2018). By limiting sea urchin recruitment, ocean warming is thought to be a driver of ecological change in Norway, as the southern boundary (65° 70' N; Fagerli et al., 2013) between kelp-dominated habitat and overgrazed urchin barren grounds continues to shift northward with corresponding increases in water temperature (Rinde et al., 2014).

Differences in thermal tolerance among lineages of *S. droebachiensis* may explain the habitat segregation we observed in the northwest Atlantic. The extreme rarity of *S. droebachiensis e* in the shallow habitat along the coast of Nova Scotia could be driven by seasonally warmer water temperatures resulting in recruitment failure, post-settlement mortality, or mortality of juveniles or adults. While summer ocean temperatures along the coast of Nova Scotia are impacted by the Gulf Stream and storm activity (Scheibling et al., 2013), lower water temperatures in the Gulf of

517 St Lawrence and coastal Newfoundland and Labrador are moderated by the cool south flowing
518 Labrador Current. The increased abundance of *S. droebachiensis e* (and *S. pallidus*) at depths
519 <15 m throughout this part of the range (i.e., north of Nova Scotia) may be explained by seasonal
520 temperatures at or below the 10°C threshold observed in the northeast Atlantic. The influence of
521 temperature on the distribution of *S. droebachiensis e* is supported by both the decrease in
522 recruitment success along the coast of Norway (Fagerli et al., 2013) and the shifting population
523 dynamics of green sea urchins in Oslofjord along the southern coast of Norway. In a response to
524 increased sea surface temperatures (SST), Nyhagen et al. (2018) demonstrated a significant shift
525 in population density from 10-15m to cooler water at 20m, and a reduction in both sea urchin
526 size and recruitment success in 1979 and 1992 compared to 2013. Additionally, while sea
527 urchins are present throughout southern Norway, abundant populations typically only occur at
528 depths of 20m or greater (e.g. site ND2 in this study; Nordberg et al., 2016). Although changes
529 in coastal SST indicate a rapid warming trend in both the northwest Atlantic (~1.0°C per decade)
530 and the margins of Norwegian and North Seas (between ~0.3 and 0.7°C per decade), particularly
531 during the planktonic dispersal and settlement of sea urchins from late spring to autumn (Lima &
532 Wethey, 2012), the samples analysed in our study were collected over a relatively short time
533 scale (1999-2015) and are unlikely to have captured ongoing changes in sea urchin distribution
534 in response to increasing SST (e.g., Hobday & Pecl, 2014). Though we suggest that temperature
535 may be an important factor in defining the range of *S. droebachiensis e*, comparative analyses of
536 the thermal tolerance of larvae, juveniles and adults of both species are required to test this
537 hypothesis.
538

539 The evolution of gamete recognition molecules has long been viewed as an important driver of
540 speciation in marine invertebrates (Vacquier, 1998; Palumbi, 2009; Lessios, 2011; Vacquier &
541 Swanson, 2011). Interspecific sperm competition in the plankton is mediated by a variety of
542 proteins and carbohydrates (sulfated polysaccharides) coating the sperm and eggs (Biermann et
543 al., 2004), and positive selection detected at sperm Bindin (e.g., Biermann, 1998) correlates with
544 the strength of reproductive isolation between species (Zigler et al., 2005). Palumbi & Lessios
545 (2005) showed that, in addition to a steady accumulation of genome divergence over time, the
546 rate of speciation in sea urchins also depends on the rate of evolution of gamete recognition
547 proteins. In their study, Palumbi & Lessios (2005) surveyed species in eight genera and showed
548 that the presence of sympatric species was common in genera with rapid evolution of sperm
549 Bindin. Since studies of Bindin evolution within *Strongylocentrotus* only included samples of *S.*
550 *droebachiensis* from the Pacific (Biermann, 1998; Pujolar & Pogson, 2011), it is difficult to
551 assess patterns of positive selection and sequence divergence between the cryptic species of *S.*
552 *droebachiensis*. However, Marks et al. (2008) detected 1.5% sequence divergence at sperm
553 Bindin between samples *S. droebachiensis* from Norway, the northwest Atlantic, and northeast
554 Pacific, and based on the conclusions of our study we suggest that this difference represents
555 interspecific divergence. In a series of heterospecific and conspecific crosses between *S.*
556 *pallidus* and *S. droebachiensis* from the Pacific and *S. droebachiensis* from Norway, Biermann
557 & Marks (2000) demonstrated strong asymmetry in fertilization compatibility among allopatric
558 populations. Our data suggests that the allopatric populations studied by Biermann & Marks
559 (2000) represent distinct species, where eggs of *S. droebachiensis e* cannot be fertilized by sperm
560 from *S. pallidus,* and we interpret their results as a test for reproductive isolation among the
561 species. Consistent with Strathmann (1981), eggs of *S. droebachiensis w* were receptive to

562 sperm from both *S. pallidus* and *S. droebachiensis e*, but sperm from *S. droebachiensis w* either
563 failed (*S. pallidus*) or had very low (*S. droebachiensis e*) fertilization rates in heterospecific
564 crosses. Similarly, eggs of *S. droebachiensis e* could not be fertilized by *S. pallidus* sperm, and
565 their sperm successfully fertilized eggs of *S. droebachiensis w* but were not tested with eggs of *S.*
566 *pallidus*. In addition to ecological segregation, patterns of sperm Bindin evolution and
567 asymmetry in reproductive compatibility may contribute to the maintenance of species
568 boundaries in sympatry in the northwest Atlantic. Detailed studies of gamete recognition
569 molecules and sperm competition among all three species will help to further understand
570 mechanisms driving evolution within the genera.
571

572 ## Conclusion

573 While previous work identified a reproductively isolated cryptic lineage of *S. droebachiensis* in
574 the northwest Atlantic (Addison & Kim, 2018), this study supports the hypothesis that these
575 species formed as a result of vicariant speciation driven by trans-Arctic isolation. Our results
576 show widespread sharing of *S. pallidus* and *S. droebachiensis w* haplotype variants throughout
577 the north Pacific and north Atlantic Oceans, but that *S. droebachiensis e* is largely restricted to
578 the north Atlantic. We detected low genetic subdivision between *S. droebachiensis w* from the
579 north Pacific and the Labrador Sea, suggesting widespread trans-Arctic gene flow in this species.
580 There was weaker evidence of trans-Arctic dispersal in *S. pallidus*, which could possibly be an
581 artefact of poor sampling of this species in the Pacific. Our analyses of biogeography and *COI*
582 sequence diversity suggests that following allopatric speciation during the Pliocene or early
583 Pleistocene, these species established a zone of secondary contact in the northwest Atlantic and
584 the Labrador Sea. In the northwest Atlantic, we identified sites along the coast of Labrador

(NAI) and Nova Scotia (OWH) where all three species of *Strongylocentrotus* are abundant, providing a natural laboratory for studying the ecological and molecular aspects driving the evolution of barriers to gene exchange. From a biogeographic perspective, understanding the mechanisms shaping the distribution of the *S. droebachiensis* species throughout the north Atlantic requires experiments to determine the physiological limits of both. We observed patterns of ecological segregation among the species that suggest temperature may play a role in habitat selection, particularly in the warmer water along the coast of Nova Scotia. In addition, while Addison & Kim (2018) provided evidence of reproductive isolation among species collected from Nova Scotia, a wider study aimed at detecting hybridization and introgression at nuclear loci is required to characterize the extent of reproductive isolation across a broader range of habitats. Viewed with a species-specific lens, both rapid sequence divergence at sperm Bindin (Marks et al., 2008) and the accumulation of interspecific gamete incompatibility (Biermann & Marks, 2000) between *S. droebachiensis w* and *S. droebachiensis e* suggests a potential role of reinforcement selection for pre-zygotic isolation (Coyne & Orr, 2004) following secondary contact. By characterizing the extent of reproductive isolation, both laboratory studies of sperm competition and interspecific fertilization combined with analyses of molecular evolution at gamete recognition loci will help to identify mechanisms that drive barriers to gene exchange in natural populations.

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612 **References:**
613

614 Addison JA, and Hart MW. 2004. Analysis of population genetic structure of the green sea
615 urchin (*Strongylocentrotus droebachiensis*) using microsatellites. Marine Biology, 144:
616 243–251. DOI: 10.1007/s00227-003-1193-6
617 Addison JA, and Hart MW. 2005. Colonization, dispersal, and hybridization influence
618 phylogeogra- phy of North Atlantic sea urchins (Strongylocentrotus droebachiensis).
619 Evolution, 59: 532–543. PMID: 15856696 DOI: 10.1554/04-238
620 Addison JA and Kim J-H. 2018. Cryptic species diversity and reproductive isolation among
621 sympatric lineages of Strongylocentrotus sea urchins in the northwest Atlantic. FACETS
622 3: 61–78. doi:10.1139/facets-2017-0081
623 Addison JA, and Pogson GH. 2009. Multiple gene genealogies reveal asymmetrical
624 hybridization and introgression among strongylocentrotid sea urchins. Molecular
625 Ecology, 18: 1239–1251. PMID: 19222750 DOI: 10.1111/j.1365-294X.2009.04094.x
626 Andrews, A.J., Christiansen, J.S., Bhat, S, Lynghammar A, Westgaard J-I, Pampoulie C, an
627 Præbel K. 2019. Boreal marine fauna from the Barents Sea disperse to Arctic Northeast
628 Greenland. *Sci Rep* 9, 5799. https://doi.org/10.1038/s41598-019-42097-x
629 Balch T, and Scheibling RE. 2000. Temporal and spatial variability in settlement and
630 recruitment of echinoderms in kelp beds and barrens in Nova Scotia. Marine Ecology
631 Progress Series. 205:139-154.

632 Berggren, W. A., and C. D. Hollister. 1974. Paleogeography, paleobio- geography, and the
633 history of circulation in the Atlantic Ocean. *Soc. Econ. Paleontol. Mineral.* 20: 126–186
634 Biermann CH. 1998. The molecular evolution of sperm bindin in six species of sea urchins
635 (Echinoida: Strongylocentrotidae). Molecular Biology and Evolution, 15(12): 1761–
636 1771. PMID: 9866210 DOI: 10.1093/oxfordjournals.molbev.a025902
637 Biermann CH, and Marks JA. 2000. Geographic divergence of gamete recognition systems in
638 two species of the sea urchin genus Strongylocentrotus. Zygote, 8: S86–S87. PMID:
639 11191337
640 Biermann CH, Marks JA, Vilela-Silva A-CES, Castro MO, and Mourão PAS. 2004.
641 Carbohydrate- based species recognition in sea urchin fertilization: another avenue for
642 speciation? Evolution & Development, 6(5): 353–361. PMID: 15330868 DOI:
643 10.1111/j.1525-142X.2004.04043.x
644 Bluhm BA, Piepenburg D, and von Juterzenka K. 1998. Distribution, standing stock, growth,
645 mortality and production of Strongylocentrotus pallidus (Echinodermata: Echinoidea) in
646 the northern Barents Sea. Polar Biology, 20:325-334.
647 Bradbury IR, Hubert S, Higgins B, Borza T, Bowman S, Paterson IG, Snelgrove PV, Morris CJ,
648 Gregory RS, Hardie DC, Hutchings JA, Ruzzante DE, Taggart CT, Bentzen P. 2010
649 Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in
650 response to temperature. Proc Biol Sci. 277(1701):3725-34. doi:
651 10.1098/rspb.2010.0985.
652 Bringloe TT. and Saunders GW. 2019. DNA barcoding of the marine macroalgae from Nome,
653 Alaska (Northern Bering Sea) reveals many trans-Arctic species. Polar Biology, 42: 851-
654 864. doi.org/10.1007/s00300-019-02478-4

655 Bringloe TT, Verbruggen H, Saunders GW (2020) Unique biodiversity in Arctic marine forests
656 is shaped by diverse recolonisation pathways and far northern glacial refugia. Proc Natl
657 Acad Sci 117:22590–22596
658 Carr CM, Hardy SM, Brown TM, Macdonald TA, Hebert PDN (2011) A Tri-Oceanic
659 Perspective: DNA Barcoding Reveals Geographic Structure and Cryptic Diversity in
660 Canadian Polychaetes. PLoS ONE 6(7): e22232. doi:10.1371/journal.pone.0022232
661
662 Chevolot, M., Hoarau, G., Rijnsdorp, A. D., Stam, W. T., & Olsen, J. L. (2006). Phylogeography
663 and population structure of thornback rays (*Raja clavata* L., Rajidae). *Molecular*
664 *Ecology*, *15*(12), 3693–3705. https://doi.org/10.1111/j.1365-294X.2006.03043.x
665
666 Clement M, Posada D, and Crandall KA. 2000. TCS: a computer program to estimate gene
667 genealogies. Molecular Ecology, 9: 1657–1659. PMID: 11050560 DOI: 10.1046/j.1365-
668 294x.2000.01020.x
669 Coyne JA, and Orr HA. 2004. Speciation. Sinauer Associates, Sunderland, Massachusetts.
670 Cronin, T. M. 1988. Evolution of marine climates of the U. S. Atlantic coast during the past four
671 million years. *Philos. Trans. R. Soc. Lond. B* 318: 661–678.
672 Cunningham, C. W., & Collins, T. M. (1998). Beyond area relationships: Extinction and
673 recolonization in molecular marine biogeography. *Molecular approaches to ecology and*
674 *evolution* (pp. 297–321). Basel, Switzerland: Birkhäuser Verlag.
675 Danielssen DS, Svendsen E, and Ostrowski M. 1996. Long-term hydrographic variation in the
676 Skagerrak based on the section Torungen–Hirtshals. ICES Journal of Marine Science,
677 53:917-925.

678 Durham J, MacNeil F: Cenozoic migrations of marine invertebrates through the Bearing Strait
679 region. In The Bering Land Bridge. Edited by Hopkins D. Palo Alto, CA: Stanford
680 University Press; 1967:326–349.
681 Edmands S, Moberg PE, and Burton RS. 1996. Allozyme and mitochondrial DNA evidence of
682 population subdivision in the purple sea urchin *Strongylocentrotus purpuratus*. Marine
683 Biology, 126: 443–450. DOI: 10.1007/BF00354626
684 Einarsson, T., Hopkins, D. M. & Doell, R. D. 1967. in The Bering Land Bridge (ed. Hopkins, D.
685 M.) 312-325 (Stanford Univ. Press).
686 Excoffier L, and Lischer HE. 2010. ARLEQUIN suite ver 3.5: a new series of programs to
687 perform population genetics analyses under Linux and Windows. Molecular Ecology
688 Resources, 10: 564–567. PMID:21565059. doi:10.1111/j.1755-0998.2010.02847.x.
689 Fagerli CW, Norderhaug KM, Christie HC (2013) Lack of sea urchin settlement may explain
690 kelp forest recovery in overgrazed areas in Norway. Mar Ecol Prog Ser 488:119-
691 132. https://doi.org/10.3354/meps10413
692 Faria, R, Johannesson, K, Stankowski, S. 2021. Speciation in marine environments: Diving
693 under the surface. *J Evol Biol*. 34: 4– 15. https://doi.org/10.1111/jeb.13756
694 Filbee-Dexter K, Scheibling RE. 2014. Detrital kelp subsidy supports high reproductive
695 condition of deep-living sea urchins in a sedimentary basin. Aquatic Biology. 23: 71-86.
696 doi: 10.3354/ab00607.
697 Fitzpatrick, B. (2009). Power and sample size for nested analysis of molecular
698 variance. *Molecular Ecology,* 18:3961-3966.

699 Franz, D. R., E. K. Worley, and A. S. Merrill. 1981. Distribution patterns of common seastars of
700 the middle Atlantic continental shelf of the northwest Atlantic (Gulf of Maine to Cape
701 Hatteras). *Biol. Bull.* 160: 394–418.
702 Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking
703 and background selection. *Genetics*. 147(2):915-25. PMID: 9335623; PMCID:
704 PMC1208208.
705 Gagnon JM, and Gilkinson KD. 1994. Discrimination and distribution of the sea urchins
706 Strongylocentrotus droebachiensis (O.F. Müller) and S. pallidus (G.O. Sars) in the
707 Northwest Atlantic. Sarsia, 79: 1–11. DOI: 10.1080/00364827.1994.10413542
708 Gladenkov, A. Y., Oleinik, A. E., Marincovich, L., & Barinov, K. B. 2002 A refined age for the
709 earliest opening of Bering Strait. *Palaeogeography Palaeoclimatology Palaeoecology*,
710 *183*, 321–328. https://doi. org/10.1016/S0031-0182(02)00249-3
711 Government of Canada. 2014. Fisheries and Oceans Canada. http:// www.pac.dfo-
712 mpo.gc.ca/science/oceans/data-donnees/lighthouses- phares/index-eng.htm.
713 Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, and Gascuel O. 2010. New
714 algorithms and methods to estimate maximum-likelihood phylogenies: assessing the
715 performance of PHYML 3.0. Systematic Biology, 59: 307–321. PMID: 20525638 DOI:
716 10.1093/sysbio/syq010
717 Harper, F. M., and M. W. Hart. 2005. Gamete compatibility and sperm com- petition affect
718 paternity and hybridization between sympatric *Asterias* sea stars. Biol. Bull. 209:113–
719 126.

720 Harper, F. M., and M. W. Hart. 2007. Morphological and phylogenetic evidence for
721 hybridization and introgression in a sea star secondary contact zone. Invertebr. Biol.
722 124:373-384.
723 Harper FM, Addison JA, and Hart MW. 2007. Introgression versus immigration in hybridizing
724 high- dispersal echinoderms. Evolution, 61: 2410–2418. PMID: 17714502 DOI:
725 10.1111/j.1558-5646. 2007.00200.x
726 Harris SA. 2005. Thermal history of the Arctic Ocean environs adjacent to North America
727 during the last 3.5 Ma and a possible mechanism for the cause of the cold events (major
728 glaciations and permafrost events). *Progress in Physical Geography* 29(2):1–19
729 Hart MW, Byrne M and SL Johnson. 2003. *Patiriella pseudoexigua* (Asteroidea: Asterinidae): a
730 cryptic species complex revealed by molecular and embryological analyses. Journal of
731 the Marine Biological Association of the United Kingdom 83: 1109-1116.
732 Hart MW, Scheibling RE (1988) Heat waves, baby booms, and the destruction of kelp beds by
733 sea urchins. Mar Biol 99:167–176
734 Haug, G. H., Sigman, D. M., Tiedemann, R., Pedersen, T. F. & Sarnthein, M. 1999. Onset of
735 permanent stratification in the subarctic Pacific Ocean. *Nature* 401, 779–782.
736 Herman, Y. & Hopkins, D. M. 1980. Arctic oceanic climate in Late Cenozoic time. Science 209,
737 557-562.
738 Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and
739 speciation. Biol. J. Linn. Soc. 58: 247–276.
740 Hoarau, G., Coyer, J. A., Veldsink, J. H., Stam, W. T., & Olsen, J. L. (2007). Glacial refugia and
741 recolonization pathways in the brown seaweed *Fucus serratus*. *Molecular Ecology*,
742 *16*(17), 3606–3616.

743 Hobday, A.J., Pecl, G.T. 2014. Identification of global marine hotspots: sentinels for change
744 and vanguards for adaptation action. *Rev Fish Biol Fisheries* **24,** 415–425.
745 https://doi.org/10.1007/s11160-013-9326-6
746 Horikawa, K., Martin, E., Basak, C., Onodera J, Seki O, Sakamoto T, Ikehara M, Sakai S, and
747 Kawamura K. 2015. Pliocene cooling enhanced by flow of low-salinity Bering Sea
748 water to the Arctic Ocean. *Nat Commun* **6,** 7587. https://doi.org/10.1038/ncomms8587
749 Hrincevich AW, Rocha-Olivares A and DW Foltz. 2000. Phylogenetic analysis of molecular
750 lineages in a species-rich subgenus of sea stars *(Leptasterias* subgenus *Hexasterias).*
751 American Zoologist 40: 365-374.
752 Ibrahim, A Olsen A, Lauvset S, and Rey F. 2014. Seasonal Variations of the Surface Nutrients
753 and Hydrography in the Norwegian Sea" *International Journal of Environmental Science*
754 *and Development* 5(5): 496-505.
755 Jacobs, H. T., D. J. Elliot, V. B. Math, and A. Farquharson. 1988. Nucleotide sequence and gene
756 organization of sea urchin mi- tochondrial DNA. J. Mol. Biol. 202:185–217.
757 Jeffery N.W., Stanley, R.R.E, Wringe, B.F., Guijarro-Sabaniel, J., Bourret, V., Bernatchez, L.,
758 Bentzen, P., Beiko, R.G., Gilbey, J., Clément, M., and Bradbury, I.R. 2017 Range-wide
759 parallel climate-associated genomic clines in Atlantic salmon. *R. Soc. open sci.* **4**:
760 171394. http://dx.doi.org/10.1098/rsos.171394
761 Jensen M. 1974. The Strongylocentrotidae (Echinoidea), a morphologic and systematic study.
762 Sarsia, 57: 113–148. DOI: 10.1080/00364827.1974.10411273
763 Jesus FF, Wilkins JF, Solferini VN, Wakeley J. 2006. Expected coalescence times and
764 segregating sites in a model of glacial cycles. *Genet Mol Res*. 5(3):466-74. PMID:
765 17117361.

766 Jolly, M. T., Viard, F., Gentil, F., Thiébaut, É., & Jollivet, D. (2006). Comparative
767 phylogeography of two coastal polychaete tubeworms in the Northeast Atlantic supports
768 shared history and vicariant events. *Molecular Ecology*, *15*(7), 1841–1855.
769 https://doi.org/10.1111/j.1365-294X.2006.02910.x
770 Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions
771 through comparative studies of nucleotide sequences. Journal of Molecular Evolution.
772 16:111-120.
773 Kumar S, Stecher G, Li M, Knyaz C, and Tamura K. 2018. MEGA X: Molecular Evolutionary
774 Genetics Analysis across computing platforms. Molecular Biologuy and Evolution.
775 35:1547-1549.
776 Kober KM, and Bernardi G. 2013. Phylogenomics of strongylocentrotid sea urchins. BMC
777 Evolutionary Biology, 13: 88. PMID: 23617542 DOI: 10.1186/1471-2148-13-88
778 Laakkonen HM, Hardman M, Strelkov P, and Väinölä R. 2021. Cycles of trans-Arctic dispersal
779 and vicariance, and diversification of the amphi-boreal marine fauna. *J Evol Biol.*
780 2021;34:73–96.
781 Layton KKS, Corstorphine EA, and Hebert PDN. 2016. Exploring Canadian echinoderm
782 diversity through DNA barcodes. PLoS ONE, 11(11): e0166118. PMID: 27870868 DOI:
783 10.1371/journal. pone.0166118
784 Ledu D, Rochon A, de Vernal A, and St-Onge G. 2008. Palynological evidence of Holocene
785 climate change in the eastern Arctic: a possible shift in the Arctic oscillation at the
786 millennial time scale. *Canadian Journal of Earth Sciences*. **45**(11): 1363-
787 1375. https://doi.org/10.1139/E08-043

788 Lessios HA. 2011. Speciation genes in free-spawning marine invertebrates. Integrative &
789 Comparative Biology, 51(3): 456–465. PMID: 21700571 DOI: 10.1093/icb/icr039
790 Librado P, and Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA
791 polymor- phism data. Bioinformatics, 25: 1451–1452. PMID:19346325.
792 doi:10.1093/bioinformatics/btp187.
793 Lima, F., Wethey, D. 2012. Three decades of high-resolution coastal sea surface temperatures
794 reveal more than warming. Nat Commun 3, 704. https://doi.org/10.1038/ncomms1713
795 Lisiecki, LE, and Raymo, ME. 2005. A Pliocene-Pleistocene stack of 57 globally distributed
796 benthic δ18O records. *Paleoceanography*, *20*, PA1003.
797 Loeza-Quintana T, Carr CM, Khan T, Bhatt YA, Lyon SP, Hebert PDN, and Adamowicz SJ.
798 2019. Recalibrating the molecular clock for Arctic marine invertebrates based on DNA
799 barcodes. *Genome*.**62**(3): 200-216. https://doi.org/10.1139/gen-2018-0107
800 Maggs, C.A., Castilho, R., Foltz, D., Henzler, C., Jolly, M.T., Kelly, J., Olsen, J., Perez, K.E.,
801 Stam, W., Väinölä, R., Viard, F. and Wares, J. (2008). Evaluating signatures of glacial
802 refugia for North Atlantic benthic marine taxa. *Ecology*, *89*(sp11), S108–S122. https://
803 doi.org/10.1890/08-0257.1
804 Manier MK, and Palumbi SR. 2008. Intraspecific divergence in sperm morphology of the green
805 sea urchin, Strongylocentrotus droebachiensis: implications for selection in broadcast
806 spawners. BMC Evolutionary Biology, 8: 283. PMID: 18851755 DOI: 10.1186/1471-
807 2148-8-283
808 Marincovich, L., & Gladenkov, A. Y. (2001). New evidence for the age of Bering Strait.
809 *Quaternary Science Reviews*, *20*, 329–335. https://doi. org/10.1016/S0277-
810 3791(00)00113-X

811 Marks JA, Biermann CH, Eanes WF, and Kryvi H. 2008. Sperm polymorphism within the sea
812 urchin Strongylocentrotus droebachiensis: divergence between Pacific and Atlantic
813 oceans. The Biological Bulletin, 215: 115–125. PMID: 18840772 DOI:
814 10.2307/25470692
815 Maslin MA, Haug GH, Sarnthein S, Tiedemann R. 1996. The progressive intensification of
816 northern hemisphere glaciation as seen from the North Pacific. Geol Rundsch (1996)
817 85:452–465.
818 Miglietta MP, Faucci A, Santini F. 2011. Speciation in the Sea: Overview of the Symposium
819 and Discussion of Future Directions, *Integrative and Comparative Biology*, 51(3): 449–
820 455, https://doi.org/10.1093/icb/icr024
821 Müller, O.F. (1776). *Zoologiæ Danicæ Prodromus, seu Animalium Daniæ et Norvegiæ*
822 *indigenarum characteres, nomina, et synonyma imprimis popularium*. Havniæ
823 [Copenhagen]: Hallageri. xxxii + 274 pp. http://www.biodiversitylibrary.org/item/47550
824 Neiva J, Serrão EA, Paulino C, Gouveia L, Want A, Tamigneaux E, Ballenghien M, Mauger S,
825 Fouqueau L, Engel-Gautier C, Destombe C, and Valero M. 2020. Genetic structure of
826 amphi-Atlantic Laminaria digitata (Laminariales, Phaeophyceae) reveals a unique range-
827 edge gene pool and suggests post-glacial colonization of the NW Atlantic, European
828 Journal of Phycology, 55:4, 517-528, DOI: 10.1080/09670262.2020.1750058
829 Neiva, J., Paulino, C., Nielsen, M.M., Krause-Jensen D, Saunders GW, Assis J, Bárbara I,
830 Tamigneaux E, Gouveia L, Aires T, Marbà N, Bruhn A, Pearosn GA, Serrão EA. 2018.
831 Glacial vicariance drives phylogeographic diversification in the amphi-boreal
832 kelp *Saccharina latissima*. *Sci Rep* 8, 1112 (2018). https://doi.org/10.1038/s41598-018-
833 19620-7

834 Norderhaug KM, Anglès d'Auriac MB, Fagerli CW, Gundersen H, Christie H, Dahl K, et al.
835 2016. Genetic diversity of the NE Atlantic sea urchin Strongylocentrotus droebachiensis
836 unveils chaotic genetic patchiness possibly linked to local selective pressure. Marine
837 Biology, 163: 36. PMID: 26843658 DOI: 10.1007/s00227-015-2801-y
838 Nyhagen, F.O., Christie, H., & Norderhaug, K.M. (2018). Will altered climate affect a discrete
839 population of the sea urchin Strongylocentrotus droebachiensis. *Journal of Sea Research,*
840 *132*, 24-34.
841 Palumbi SR. 2009. Speciation and the evolution of gamete recognition genes: pattern and
842 process. Heredity, 102: 66–76. PMID: 19018273 DOI: 10.1038/hdy.2008.104
843 Palumbi SR, Grabowsky G, Duda T, Geyer L and N Tachino. 1997. Speciation and population
844 genetic structure in tropical Pacific sea urchins. Evolution 51: 1506-1517.
845 Palumbi, S. R., and B. D. Kessing. 1991. Population biology of the trans-Arctic exchange:
846 mtDNA sequence similarity between Pacific and Atlantic sea urchins. Evolution
847 45:1790–1805.
848 Palumbi SR, Lessios HA. 2005. Evolutionary animation: how do molecular phylogenies
849 compare to Mayr's reconstruction of speciation patterns in the sea? Proc Natl Acad Sci U
850 S A. 3;102 Suppl 1(Suppl 1):6566-72. doi: 10.1073/pnas.0501806102. Epub 2005 Apr
851 25. PMID: 15851681; PMCID: PMC1131860.
852 Palumbi, S. R., and A. C. Wilson. 1990. Mitochondrial DNA diversity in the sea urchins
853 *Strongylocentrotus purpuratus* and *S. droebachiensis*. Evolution 44:403–415.
854 Payne MC, Brown CA, Reusser DA, Lee H II. 2012. Ecoregional Analysis of Nearshore Sea-
855 Surface Temperature in the North Pacific. PLoS ONE 7(1): e30105.
856 doi:10.1371/journal.pone.0030105

857 Pearce CM, Williams SW, Yuan F, Castell JD, and Robinson SM. 2005. Effect of temperature
858 on somatic growth and survivorship of early post-settled green sea urchins,
859 Strongylocentrotus droebachiensis (Müller). Aquaculture Research, 36: 600-609
860 doi:10.1111/j.1365-2109.2005.01264.x
861 Provan, J., Wattier, R. A., & Maggs, C. A. (2005). Phylogeographic analysis of the red seaweed
862 Palmaria palmata reveals a Pleistocene marine glacial refugium in the English Channel.
863 *Molecular Ecology*, *14*(3), 793–803. https://doi.org/10.1111/j.1365-294X.2005.02447.x
864 Pujolar JM, and Pogson GH. 2011. Positive Darwinian selection in gamete recognition proteins
865 of Strongylocentrotus sea urchins. Molecular Ecology, 20: 4968–4982. PMID: 22060977
866 DOI: 10.1111/ j.1365-294X.2011.05336.x
867 Ramos-Onsins SE, and Rozas J. 2002. Statistical properties of new neutrality tests against
868 population growth. Molecular Biology and Evolution, 19: 2092–2100. PMID:12446801.
869 doi:10.1093/ oxfordjournals.molbev.a004034.
870 Raymond B.G. & Scheibling R.E. (1987) Recruitment and growth of the sea urchin
871 Strongylocentrotus droebachiensis (Müller) following mass mortalities off Nova Scotia,
872 Canada. Journal of Experimental Marine Biology and Ecology 108: 31-54.
873 Rinde E, Christie H, Fagerli CW, Bekkby T, Gundersen H, et al. (2014) The Influence of
874 Physical Factors on Kelp and Sea Urchin Distribution in Previously and Still Grazed
875 Areas in the NE Atlantic. PLoS ONE 9(6): e100222. doi:10.1371/journal.pone.0100222
876 Scheibling RE, Feehan CJ, Lauzon-Guay JS (2013) Climate change, disease and the dynamics of
877 a kelp-bed ecosystem in Nova Scotia. Climate Change: Perspectives from the Atlantic:
878 Past, Present and Future, eds Fernández-Palocios JM, et al. (Servicio de Publicaciones de
879 la Universidad de La Laguna, Tenerife, Canary Islands), pp 41–81

880 Scheibling, R. E., & Raymond, B. G. (1990). Community dynamics on a subtidal cobble bed
881 following mass mortalities of sea urchins. *Marine Ecology Progress Series*, *63*(2/3), 127–
882 145. http://www.jstor.org/stable/24844610
883 Scheibling RE, and Stephenson RL. 1984. Mass mortality of Strongylocentrotus droebachiensis
884 (Echinodermata: Echinoidea) off Nova Scotia, Canada. Marine Biology 78:153-164.
885 Souche, E. L., Hellemans, B., Babbucci, M., MacAoidh, E., Guinand, B., Bargelloni, L., ...
886 Volckaert, F. A. (2015). Range-wide population struc- ture of European sea bass
887 *Dicentrarchus labrax*. *Biological Journal of the Linnaean Society*, *116*(1), 86–105.
888 Stecher G, Tamura K, and Kumar S. 2020. Molecular Evolutionary Genetics Analysis (MEGA)
889 for macOS. Molecular Biology and Evolution. 37(4): 1237–1239.
890 Strathmann RR. 1978. Length of pelagic period in echinoderms with feeding larvae from the
891 northwest Pacific. Journal of Experimental Marine Biology and Ecology, 34:23–27
892 Strathmann RR. 1981. On barriers to hybridization between Strongylocentrotus droebachiensis
893 (O.F. Müller) and S. pallidus (G.O. Sars). Journal of Experimental Marine Biology and
894 Ecology, 55: 39–47. DOI: 10.1016/0022-0981(81)90091-5
895 Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA
896 polymorphism. Genetics, 105: 437–460.
897 Vacquier VD. 1998. Evolution of gamete recognition proteins. Science, 281: 1995–1998. PMID:
898 9748153 DOI: 10.1126/science.281.5385.1995
899 Vacquier VD, and Swanson WJ. 2011. Selection in the rapid evolution of gamete recognition
900 proteins in marine invertebrates. Cold Spring Harbor Perspectives in Biology, 3:
901 a002931. PMID: 21730046 DOI: 10.1101/cshperspect.a002931

902 Wares, J. P. 2001. Biogeography of *Asterias*: North Atlantic climate change and speciation. Biol.
903 Bull. 201:95–103.
904 Young, A., Torres, C., Mack, J., & Cunningham, C. (2002). Morphological and genetic evidence
905 for vicariance and refugium in Atlantic and Gulf of Mexico populations of the hermit
906 crab *Pagurus longicar‐pus*. *Marine Biology*, *140*(5), 1059–1066. https://doi.org/10.1007/
907 s00227-002-0780-2
908 Zigler KS, McCartney MA, Levitan DR, and Lessios HA. 2005. Sea urchin bindin divergence
909 predicts gamete compatibility. Evolution, 59: 2399–2404. PMID: 16396180 DOI:
910 10.1111/j.0014-3820.2005. tb00949.x

Figure 1

Sample sites of Strongylocentrotus sea urchins throughout the North Paciûc and north Atlantic oceans (see Table 1 for abbreviations)

(A) Sample sites of Strongylocentrotus sea urchins throughout the North Paciûc and north Atlantic oceans (see Table 1 for abbreviations). Pie charts represent the proportion of mtDNA haplotypes (418bp COI) belonging to each of the three lineages. (B) inset map of samples collected throughout Atlantic Canada. (C) TCS haplotype network of COI mtDNA sequences from all three lineages of Strongylocentrotus sea urchins (n = 789) included in this study. Circle area is proportionate to the number of haplotypes sequenced and the colours of each lineage match the pie charts from A and B. Node support indicated by nonparametric bootstrap (1000 replicates) and Bayesian posterior probability, respectively. Overall mean K2P distances are within each lineage is indicated in the boxes, and mean pairwise distances are indicated along the vectors.

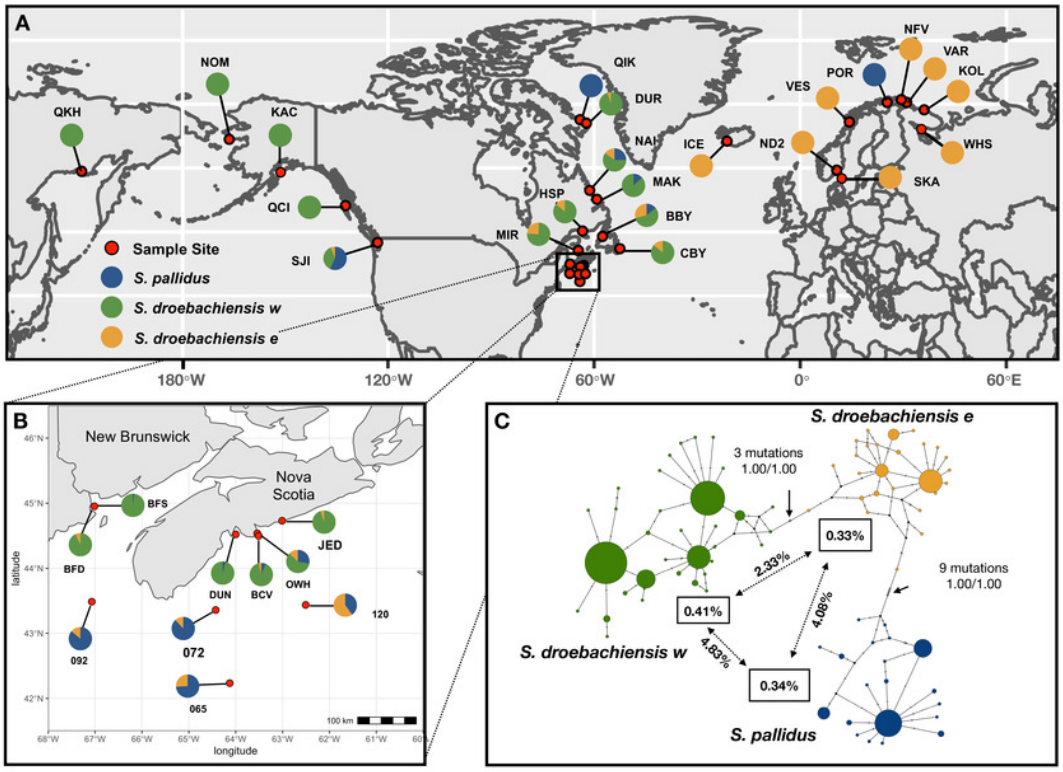
A
NOM
QKH
KAC
QCI
SJI
QIK
DUR
NAI
ICE
MAK
HSP
BBY
MIR
CBY
NFV
VAR
POR
KOL
VES
ND2
WHS
SKA
Sample Site
S. pallidus
S. droebachiensis w
S. droebachiensis e
180°W
120°W
60°W
0°
60°E
B
New Brunswick
Nova Scotia
BFS
BFD
JED
DUN
BCV
OWH
120
092
072
065
latitude
longitude
C
S. droebachiensis e
3 mutations
1.00/1.00
0.33%
2.33%
9 mutations
1.00/1.00
0.41%
4.08%
4.83%
S. droebachiensis w
0.34%
S. pallidus

Figure 2

Sampling locations, haplotype distribution, and TCS haplotype network of COI mtDNAsequences for *Strongylocentrotus pallidus* (n = 156)

(*) asterisks indicate the mtDNA haplotypes removed from analyses of population genetic structure because they were recovered in individuals whose nuclear genomes (SNPs or microsatellites) were characterized as being 100% *S. droebachiensis w* (# tested / # individuals with the haplotype).

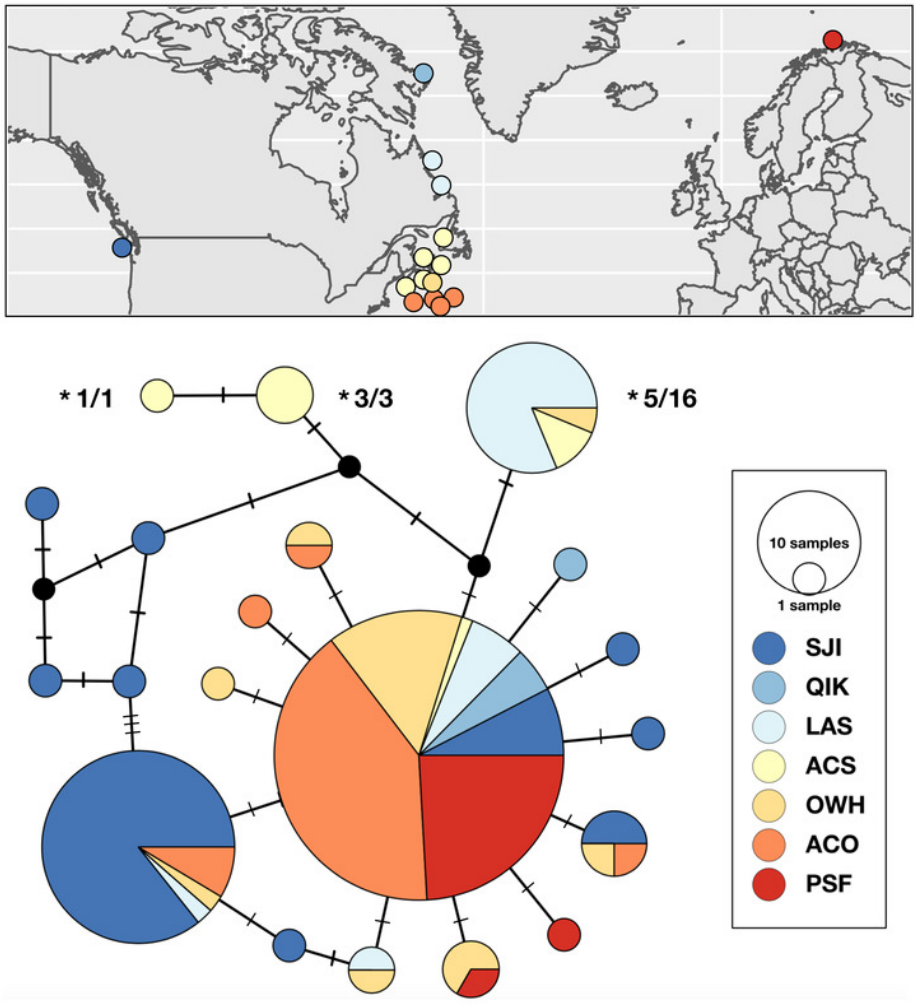

Figure 3

Sampling locations, haplotype distribution, and TCS haplotype network of *COI* mtDNA sequences for *Strongylocentrotus droebachiensis e* (n = 148)

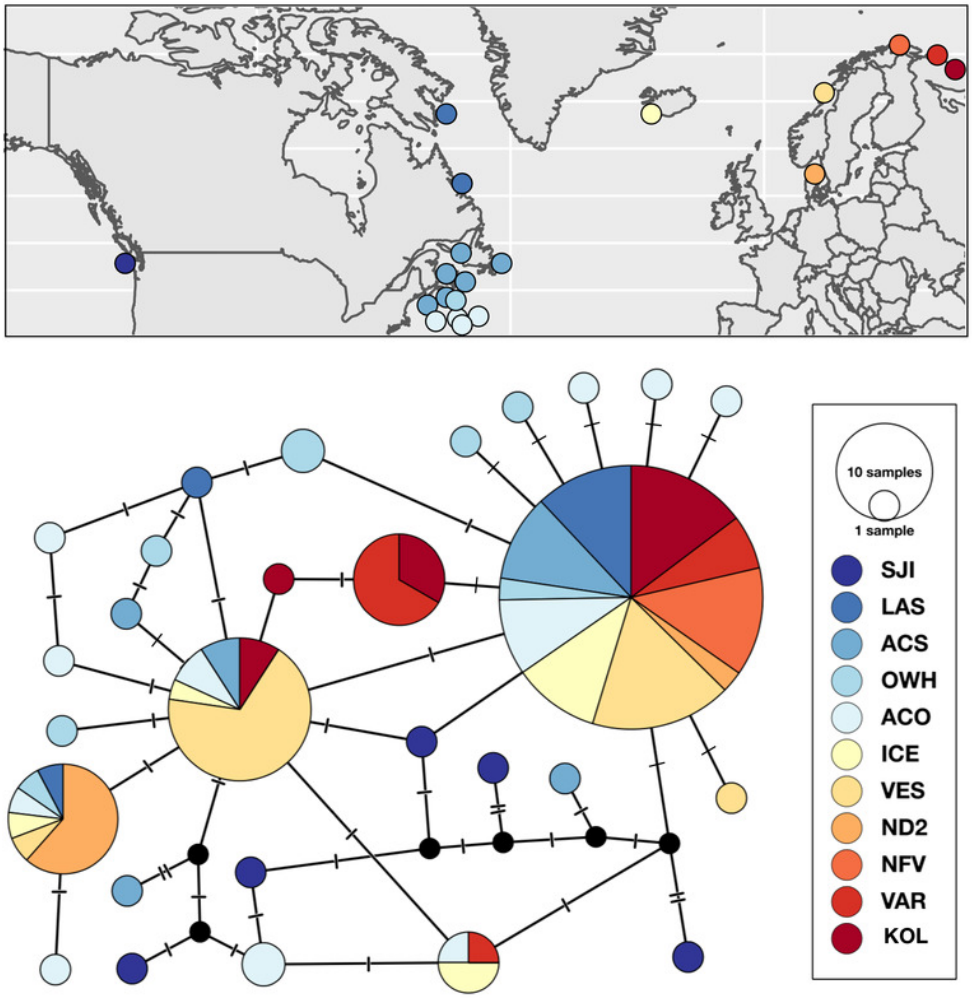

Figure 4

Sampling locations, haplotype distribution, and TCS haplotype network of *COI* mtDNA sequences for *Strongylocentrotus droebachiensis w* (n = 485)

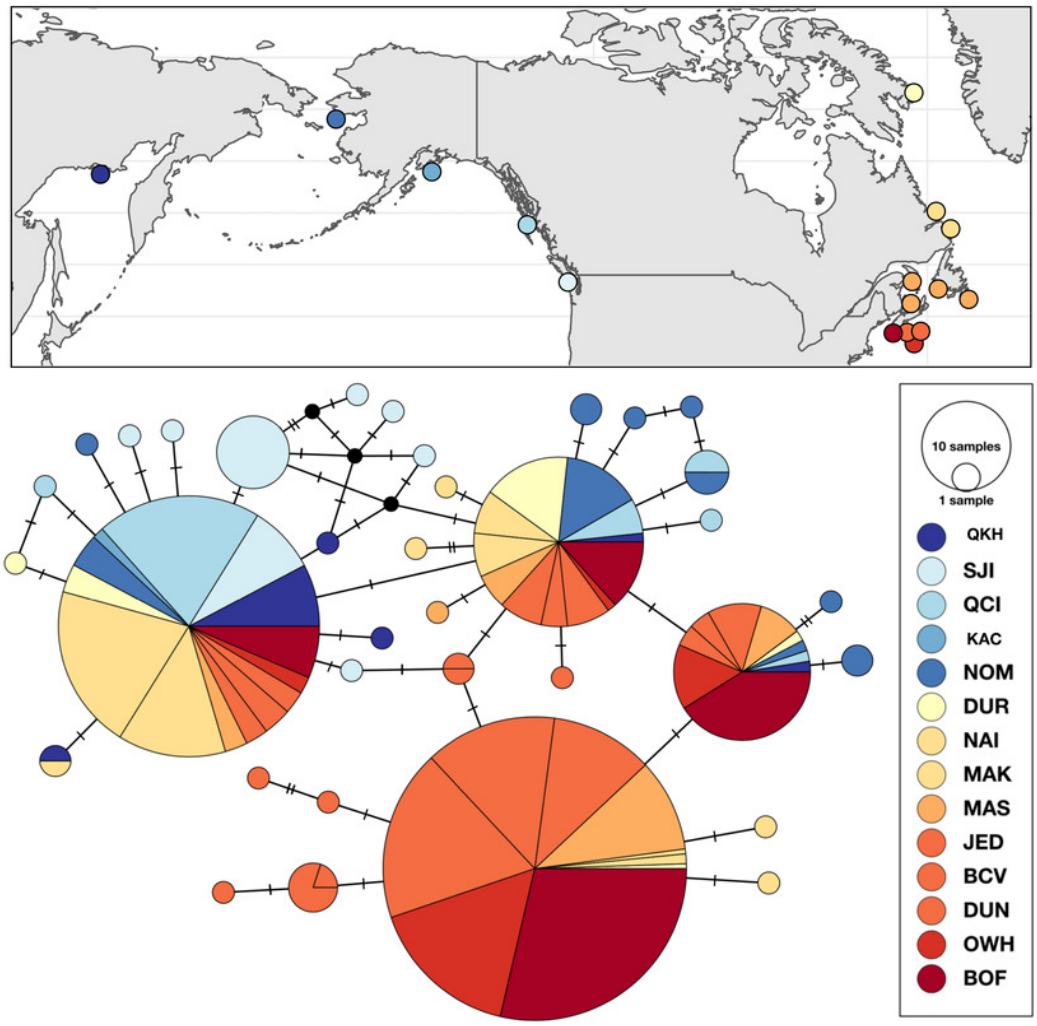

Table 1(on next page)

Sample location, site codes, sample size, sampling depth, and data sources for *Strongylocentrotus* sea urchins used in this study.

Table 1. Sample location, site codes, sample size, sampling depth, and data sources for *Strongylocentrotus* sea urchins used in this study.

Location	Abbreviation	Sample Size (N)	depth (m)	Latitude	Longitude	COI Data Source
Sea of Okhotsk, Russia	OKH	16	n/a	59.49400	-150.91500	Laakkonen et al 2021
San Juan Islands, WA	*SJI*	39	50	48.33000	-123.01000	Addison and Hart 2005; This study
San Juan Islands, WA	*SJI*	40	30	48.32800	-123.06000	Harper et al 2007; This study
Langara Island, BC	*LAN*	20	2-8	54.19300	-132.97200	This study
Massett, BC	*MAS*	18	2-8	54.10700	-132.36600	This study
Kachemak Bay, Alaska	*KAC*	2	5	59.48500	-151.64600	Laakkonen et al 2021
Nome, AK	*NOM*	26	2-8	64.48737	-166.19298	This study
Qikiqtarjuaq, NU	*QIK*	5	2-8	67.56800	-64.06700	This study
Durban Island, NU	*DUR*	19	2-8	67.03800	-62.24900	This study
Makkovik, NL	*MAK*	31	2-8	55.10200	-59.18000	This study
Nain, NL	*NAI*	65	2-8	56.50400	-61.26300	This study
Bonne Bay, NL	*BBY*	7	3-15	49.31000	-57.53000	Addison and Hart 2005
Conception Bay, NL	*CBY*	7	3-15	47.38000	-52.50000	Addison and Hart 2005
Harve Saint Pierre, QC	*HSP*	7	3-15	50.14000	-63.36000	Addison and Hart 2005
Miramichi, NB	*MIR*	21	3-15	47.08000	-64.58000	Addison and Hart 2005
Jeddore, NS	*JED*	42	3-15	44.73000	-63.01102	Addison and Hart 2005
Bear Cove, NS	*BCV*	48	3-15	44.53670	-63.54195	Addison and Hart 2005
Duncan Cove, NS	*DUN*	48	2-30	44.49794	-63.51038	Addison and Kim 2018
Owl's Head, NS	*OWH*	69	60	44.52090	-64.00069	This study
NS offshore (65m)	*065*	8	65	42.23480	-64.12820	Addison and Kim 2018
NS offshore (72m)	*072*	19	72	43.35880	-64.42790	Addison and Kim 2018
NS offshore (92m)	*092*	14	90	43.48620	-67.07020	Addison and Kim 2018
NS offshore (120m)	*120*	15	120	43.43670	-62.50380	Addison and Kim 2018

Bay of Fundy (shallow)	*BFS*	63	14	44.95233	-67.01451	This study
Bay of Fundy (deep)	*BFD*	28	70-90	44.58000	-67.00000	This study
Hvalfjordur, Iceland	*ICE*	12	10	64.21000	-21.29000	Addison and Hart 2005
Oslo fjord, Norway	*ND2*	10	20	59.66278	10.62596	This study
Skagerrak, Sweden	SKA	2	n/a	58.18000	11.47000	Laakkonen et al 2021
Vestfjorden, Norway	*VES*	28	10	67.21000	-14.30000	Addison and Hart 2005
Kongsfjord, Norway	*NFV*	10	5	70.72000	29.44000	This study
Porsangerfjorden, Norway	*PSF*	21	2-5	70.27948	25.29986	This study
Varanger Peninsula, Norway	*VAR*	12	intertidal	70.28330	30.99770	Laakkonen et al 2021
Kola Peninsula, Russia	*KOL*	16	sublittoral	69.1177	36.07680	Laakkonen et al 2021
White Sea, Russia	*WHS*	1	n/a	66.2900	33.61000	Laakkonen et al 2021

4

Table 2 (on next page)

Mitochondrial DNA (*COI*) diversity for *Strongylocentrotus* sea urchins from individual sites and within a priori groups.

Number of individuals sequenced (N), number of haplotypes (*H*), number of segregating sites (*S*), nucleotide diversity (π), haplotype diversity (*h*), and neutrality tests (Tajima's *D*; Fu's *F*). Neutrality tests significantly different from 0 after Bonferroni correction are indicated by an asterisk (*).

Table 2. Mitochondrial DNA (*COI*) diversity for *Strongylocentrotus* sea urchins from individual sites and within a priori groups. Number of individuals sequenced (N), number of haplotypes (*H*), number of segregating sites (*S*), nucleotide diversity (π), haplotype diversity (*h*), and neutrality tests (Tajima's *D*; Fu's *F*). Neutrality tests significantly different from 0 after Bonferroni correction (P < 0.0056) are indicated by an asterisk (*).

Species	Sample Site	Abbr.	Group	N	*H*	*S*		*h*	*D*	*F*
Strongylocentrotus pallidus										
	San Juan Islands, WA	SJI	SJI	45	10	10	0.0030 (0.0007)	0.544 (0.085)	-1.324	-4.43
	Qikiqtarjuaq, NU	QIK	QIK	5	2	1	0.0010 (0.0006)	0.400 (0.237)	-0.817	0.090
	Labrador Sea:		LAS	20	4	4	0.0028 (0.0005)	0.537 (0.099)	0.078	0.335
	Nain, NL	NAI		16	3	3	0.0027 (0.0005)	0.542 (0.265)	-	-
	Makkovik, NL	MAK		4	2	3	0.0036 (0.0019)	0.500 (0.104)	-	-
	Atlantic Coast Shallow:		ACS	7	4	6	0.0062 (0.0014)	0.810 (0.123)	0.254	0.354
	Bonne Bay, NL	BBY		1	1	0	0	0	-	-
	Bear Cove, NS	BCV		3	2	1	0.0016 (0.0008)	0.667 (0.314)	-	-
	Duncan Cove, NS	DUN		2	1	0	0 (0.0004)	0	-	-
	Bay of Fundy (shallow)	BFS		1	1	0	0 (0.0004)	0	-	-
	Owl's Head, NS	OWH	OWH	19	7	7	0.0020 (0.0006)	0.608 (0.127)	-1.954	-4.400*
	Atlantic Coast Offshore:		ACO	39	6	5	0.0008 (0.0003)	0.327 (0.095)	-1.800	-4.891*
	NS offshore (65m)	065		7	2	1	0.0007 (0.0005)	0.286 (0.196)	-	-
	NS offshore (72m)	072		14	3	2	0.0010 (0.0004)	0.385 (0.149)	-	-
	NS offshore (92m)	092		12	4	3	0.0012 (0.0005)	0.455 (0.170)	-	-
	NS offshore (120m)	120		6	1	0	0	0	-	-
	Porsangerfjorden, NOR	PSF	NOR	21	3	2	0.0005 (0.0003)	0.186 (0.110)	-1.514*	-1.920
	Total			**156**	**20**	**18**	**0.0033 (0.0004)**	**0.685 (0.032)**	**-1.526**	**-12.734***
Strongylocentrotus droebachiensis e										
	San Juan Islands, WA	SJI	SJI	5	5	8	0.0100 (0.0016)	1.000 (0.013)	0.477	-1.674
	Labrador Sea:		LAS	11	3	3	0.0020 (0.0008)	0.345 (0.172)	-1.113	-0.113

Durban Island, NU	DUR		1	1	0	0	0	-	-
Nain, NL	NAI		10	2	2	0.0009 (0.0007)	0.200 (0.154)	-	-
Atlantic Coast Shallow		ACS	13	5	8	0.0040 (0.0015)	0.628 (0.143)	-1.37	-0.504
Conception Bay, NL	CBY		1	1	0	0	0	-	-
Bonne Bay, NL	BBY		2	1	0	0	0	-	-
Harve Saint Pierre, QC	HSP		1	1	0	0	0	-	-
Miramichi, NB	MIR		5	1	0	0	0	-	-
Jeddore, NS	JED		2	1	0	0	0	-	-
Bear Cove, NS	BCV		2	2	5	0.0120 (0.0060)	1.000 (0.500)	-	-
Owl's Head (65m)	OWH	OWH	9	7	7	0.0051 (0.0009)	0.944 (0.070)	-0.804	-3.618
Atlantic Coast Offshore		ACO	19	11	10	0.0045 (0.0007)	0.865 (0.071)	-1.154	-6.600*
NS offshore (65m)	065		1	1	0	0	0	-	-
NS offshore (72m)	072		5	4	6	0.0062 (0.0017)	0.900 (0.161)	-	-
NS offshore (92m)	092		2	2	2	0.0048 (0.0024)	1.000 (0.500)	-	-
NS offshore (120m)	120		9	5	5	0.0033 (0.0010)	0.806 (0.120)	-	-
Bay of Fundy (deep)	BFD		2	2	1	0.0024 (0.0012)	1.000 (0.500)	-	-
Hvalfjordur, Iceland	ICE	ICE	12	4	3	0.0023 (0.0007)	0.561 (0.154)	-0.128	-0.719
Skagerrak, Sweden	SKA	ND2	2	1	1	0	0	-	-
Oslo fjord, Norway	ND2	ND2	10	2	2	0.0017 (0.0008)	0.356 (0.159)	0.019	1.532
Vestfjorden, Norway	VES	VES	28	4	3	0.0016 (0.0002)	0.587 (0.048)	-0.3387	-0.6325
Kongsfjord, Norway	NFV	NFV	10	1	0	0	0	-	-
Varanger Peninsula, Norway	VAR	VAR	12	3	3	0.0021 (0.0007)	0.621 (0.087)	-0.3785	0.4281
Kola Peninsula, Russia	KOL	KOL	16	4	2	0.0017 (0.0004)	0.592 (0.122)	0.5192	-0.9678
White Sea, Russia	WHS	KOL	1	1	0	0	0	-	-
Total			**147**	**28**	**23**	**0.0033 (0.0003)**	**0.715 (0.036)**	**-0.388**	**-0.169**
Strongylocentrotus droebachiensis w									
Sea of Okhotsk, Russia	OKH	RUS	16	6	5	0.0018 (0.0006)	0.542 (0.147)	-1.692	-3.693*
San Juan Islands, WA	SJI	SJI	29	8	9	0.0029 (0.0006)	0.702 (0.059)	-1.496	-3.277

Queen Charlotte Islands:		QCI	38	6	5	0.0014 (0.0004)	0.413 (0.097)	-1.273	-2.962
Massett, BC	MAS		18	4	3	0.0020 (0.0004)	0.595 (0.109)	-	-
Langara Island, BC	LAN		20	3	3	0.0007 (0.0005)	0.195 (0.115)	-	-
Kachemak Bay, Alaska	KAC		2	1	0	0	0	-	-
Nome, AK	NOM	NOM	26	10	9	0.0038 (0.0006)	0.834 (0.054)	-1.08	-4.832*
Durban Island, NU	DUR	DUR	18	5	4	0.0022 (0.0005)	0.641 (0.097)	-0.673	-1.521
Nain, NL	NAI	NAI	39	6	7	0.0021 (0.0006)	0.437 (0.093)	-1.336	-1.773
Makkovik, NL	MAK	MAK	27	5	5	0.0021 (0.0007)	0.484 (0.104)	-0.932	-1.123
Mid-Atlantic Shallow:		MAS	32	5	4	0.0029 (0.0004)	0.619 (0.084)	0.5347	-0.066
Conception Bay, NL	CBY		6	3	2	0.0027 (0.0012)	0.733 (0.155)	-	-
Bonne Bay, NL	BBY		4	2	3	0.0036 (0.0019)	0.500 (0.265)	-	-
Harve Saint Pierre, QC	HSP		6	2	3	0.0038 (0.0006)	0.533 (0.172)	-	-
Miramichi, NB	MIR		16	5	4	0.0028 (0.0006)	0.667 (0.113)	-	-
Jeddore, NS	JED	JED	40	9	9	0.0034 (0.0005)	0.697 (0.007)	-0.959	-2.96
Bear Cove, NS	BCV	BCV	43	7	5	0.0027 (0.0005)	0.589 (0.082)	-0.047	-1.749
Duncan Cove, NS	DUN	DUN	46	4	3	0.0021 (0.0004)	0.409 (0.085)	0.535	0.395
Owl's Head, NS	OWH	OWH	41	4	3	0.0017 (0.0005)	0.411 (0.087)	-0.011	-0.197
Bay of Fundy:		BOF	88	4	3	0.0023 (0.0003)	0.564 (0.051)	1.178	1.282
Bay of Fundy (deep)	BFD		26	3	3	0.0023 (0.0005)	0.446 (0.105)	-	-
Bay of Fundy (shallow)	BFS		62	4	3	0.0024 (0.0003)	0.605 (0.054)	-	-
Total			**485**	**33**	**27**	**0.0040 (0.0001)**	**0.733 (0.013)**	**-0.463**	**-1.729**

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Table 3(on next page)

Pairwise genetic distances (K2P) within and between oceanic regions for *Strongylocentrotus pallidus* (S.p), *S. droebachiensis e* (S.d.e), and *S. droebachiensis w* (S.d.w).

Table 3. Pairwise genetic distances (K2P) within and between oceanic regions for *Strongylocentrotus pallidus* (*S.p*), *S. droebachiensis e* (*S.d.e*), and *S. droebachiensis w* (*S.d.w*).

Ocean Basin	Species	NW Pacific	NE Pacific	NW Atlantic	NE Atlantic
NW Pacific	*S.p*	-	-	-	-
	S.d.e	-	-	-	-
	S.d.w	0.0018	0.0001	0.0025	
NE Pacific	*S.p*	-	0.0005	0.0013	0.0013
	S.d.e	-	0.0099	0.0022	0.0028
	S.d.w	-	0.0031	0.0022	
NW Atlantic	*S.p*	-	-	0.0032	0.0002
	S.d.e	-	-	0.0046	0.0001
	S.d.w	-	-	0.0034	-
NE Atlantic	*S.p*	-	-	-	0.0030
	S.d.e	-	-	-	0.0024
	S.d.w	-	-	-	-

Table 4(on next page)

Pairwise F_{ST} values among sampling locations for *Strongylocentrotus pallidus* using mitochondrial DNA (COI).

Values of F_{ST} are above the diagonal with significant values in bold, and significance after Bonferroni correction ("+" for $P < 0.0033$, "−" for $P > 0.0033$) is indicated below the diagonal. -- indicates no data.

Table 4. Pairwise F_{ST} values among sampling locations for *Strongylocentrotus pallidus* using mitochondrial DNA (*COI*). Values of F_{ST} are above the diagonal with significant values in bold, and significance after Bonferroni correction ("+" for $P < 0.0033$, "–" for $P > 0.0033$) is indicated below the diagonal. — indicates no data.

	SJI	QIK	LAS	OWH	ACO	PSF
SJI	--	0.2881	0.2023	**0.2998**	**0.3457**	**0.3704**
QIK	-	--	-0.0108	-0.0214	0.0232	0.0687
LAS	-	-	--	-0.0211	-0.0326	0.0634
OWH	+	-	-	--	0.0021	-0.0054
ACO	+	-	-	-	--	0.0076
PSF	+	-	-	-	-	--

Table 5(on next page)

Analysis of molecular variance results of mtDNA (*COI*) for three species of *Strongylocentrotus* sea urchins based on *a priori* groupings of sample sites within oceanic regions, and *ad hoc* hypotheses based on analyses of pairwise [i]F[

Significant values ($P < 0.05$) of Φ_{CT} (variation among groups), Φ_{ST} (variation within populations), and Φ_{SC} (variation among populations within groups) are in bold.

Table 5. Analysis of molecular variance results of mtDNA (*COI*) for three species of *Strongylocentrotus* sea urchins based on *a priori* groupings of sample sites within oceanic regions, and *ad hoc* hypotheses based on analyses of pairwise F_{ST}. Significant values ($P < 0.05$) of Φ_{CT} (variation among groups), Φ_{ST} (variation within populations), and Φ_{SC} (variation among populations within groups) are in bold.

Hypothesis	Grouping	Φ_{CT}	Φ_{ST}	Φ_{SC}	Φ_{CT} *P*	Φ_{ST} *P*	Φ_{SC} *P*
S. pallidus							
Among oceanic regions (Pacific/NWA/NEA)	(SJI) + (QIK, LAS, OWH, ACO) + (PSF)	0.353	**0.334**	-0.029	0.205	<0.001	0.516
Among oceanic regions, subdivision based on depth within NWA	(SJI) + (QIK, LAS) + (OWH, ACO) + (PSF)	0.321	**0.306**	-0.021	0.180	<0.001	0.446
Intra-Atlantic (NWA/NEA)	(LAS, QIK, OWH, ACO) + (PSF)	-0.006	-0.002	0.005	0.602	0.516	0.395
S. droebachiensis **e**							
Among oceanic regions (Pacific/NWA/NEA)	(SJI) + (LAS,ACS, OWH, ACO) + (ICE, ND2, VES, NFV, VAR, KOL)	0.091	**0.237**	**0.161**	0.086	<0.001	<0.001
Intra-Atlantic (NWA/NEA)	(LAS,ACS, OWH, ACO) + (ICE, VES, ND2, NFV, VAR, KOL)	-0.025	**0.156**	**0.177**	0.660	<0.001	<0.001
Within the NEA only: North Sea, Norwegian Sea, Barents Sea	(ND2) + (ICE, VES) + (NFV, VAR, KOL)	**0.329**	**0.377**	**0.072**	<0.001	<0.001	0.042

***S. droebachiensis* w**

Among oceanic regions (NWP/NEP/NWA/NEA)	(OKH) + (SJI, QCI, NOM) + (DUR, NAI, MAK, MAS, JED, BCV, OWH, BOF)	**0.314**	**0.547**	**0.340**	0.036	<0.001	<0.001
Among oceanic regions, north south subdivision in NWA	(OKH) + (SJI, QCI, NOM) + (DUR, NAI, MAK) + (MAS, JED, BCV, OWH, BOF)	**0.497**	**0.539**	**0.084**	0.001	<0.001	<0.001
Grouped by latitude	(NOM, DUR) + (KOH, QCI, NAI, MAK) + (SJI, MAS, JED, BCV, OWH, BOF)	**0.396**	**0.540**	**0.239**	0.015	<0.001	<0.001

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Table 6(on next page)

Pairwise F_{ST} values among sampling locations for *Strongylocentrotus droebachiensis e* using mitochondrial DNA (*COI*).

Values of F_{ST} are above the diagonal with significant values in bold, and significance after Bonferroni correction ("+" for $P < 0.0009$, "−" for $P > 0.0009$) is indicated below the diagonal. -- indicates no data.

Table 6. Pairwise F_{ST} values among sampling locations for *Strongylocentrotus droebachiensis e* using mitochondrial DNA (*COI*). Values of F_{ST} are above the diagonal with significant values in bold, and significance after Bonferroni correction ("+" for $P < 0.0009$, "–" for $P > 0.0009$) is indicated below the diagonal. — indicates no data.

	SJI	LAB	ACS	OWH	ACO	ICE	ND2	VES	NFV	VAR	KOL
SJI	--	**0.4549**	0.3022	**0.3175**	0.2921	**0.3850**	**0.5238**	**0.5568**	**0.5546**	**0.4795**	**0.5090**
LAB	+	--	-0.0091	-0.0025	0.0247	-0.0161	0.4852	0.0960	0.0393	0.2134	0.0333
ACS	-	-	--	0.0227	0.0046	-0.0087	**0.3529**	0.0432	0.0675	0.1691	0.047
OWH	+	-	-	--	0.0216	0.0325	0.0347	0.1089	0.1513	0.2028	0.1111
ACO	-	-	-	-	--	-0.0333	0.2122	0.0129	0.1329	0.1863	0.0898
ICE	+	-	-	-	-	--	0.3852	0.0190	0.1570	0.2143	0.0667
ND2	+	-	+	-	-	-	--	**0.4022**	**0.7142**	**0.5895**	**0.5369**
VES	+	-	-	-	-	-	+	--	0.3060	**0.3500**	0.1589
NFV	+	-	-	-	-	-	+	-	--	0.3118	0.1183
VAR	+	-	-	-	-	-	+	+	-	--	0.0277
KOL	+	-	-	-	-	-	+	-	-	-	--

Table 7 (on next page)

Pairwise F_{ST} values among sampling locations for *Strongylocentrotus droebachiensis w* using mitochondrial DNA (*COI*).

Values of F_{ST} are above the diagonal with significant values in bold, and significance after Bonferroni correction ("+" for $P < 0.0006$, "−" for $P > 0.0006$) is indicated below the diagonal. -- indicates no data.

Table 7. Pairwise F_{ST} values among sampling locations for *Strongylocentrotus droebachiensis w* using mitochondrial DNA (*COI*). Values of F_{ST} are above the diagonal with significant values in bold, and significance after Bonferroni correction ("+" for $P < 0.0006$, "–" for $P > 0.0006$) is indicated below the diagonal. — indicates no data.

	OKH	SJI	QCI	NOM	DUR	NAI	MAK	MAS	JED	BCV	DUN	OWH	BOF
OKH	--	0.1712	-0.0061	0.2227	0.2437	-0.0096	0.0107	**0.5553**	**0.5317**	**0.6197**	**0.6771**	**0.7293**	**0.6183**
SJI	-	--	**0.2316**	**0.3510**	**0.3614**	**0.2100**	**0.2164**	**0.5932**	**0.5749**	**0.6359**	**0.6877**	**0.7232**	**0.6575**
QCI	-	+	--	**0.2217**	0.2192	-0.0046	-0.0006	**0.5890**	**0.5673**	**0.6470**	**0.6922**	**0.7390**	**0.6287**
NOM	-	+	+	--	-0.0105	**0.1873**	0.1340	**0.3333**	**0.3342**	**0.4419**	**0.4836**	**0.5327**	**0.4357**
DUR	-	+	-	-	--	0.1558	0.1053	**0.3756**	**0.3613**	**0.4808**	**0.5376**	**0.6046**	**0.4641**
NAI	-	+	-	+	-	--	-0.0205	**0.5131**	**0.5009**	**0.5826**	**0.6275**	**0.6747**	**0.5735**
MAK	-	+	-	-	-	-	--	**0.4852**	**0.4702**	**0.5618**	**0.6134**	**0.6670**	**0.5547**
MAS	+	+	+	+	+	+	+	--	-0.0236	0.0119	0.0112	0.0400	-0.0116
JED	+	+	+	+	+	+	+	-	--	-0.0012	0.0038	0.0266	-0.0072
BCV	+	+	+	+	+	+	+	-	-	--	-0.0123	0.0018	0.0117
DUN	+	+	+	+	+	+	+	-	-	-	--	-0.0106	0.0034
OWH	+	+	+	+	+	+	+	-	-	-	-	--	0.0126
BOF	+	+	+	+	+	+	+	-	-	-	-	-	--