Vertical water mass structure in the North Atlantic influences the bathymetric distribution of species in the deep-sea coral genus *Paramuricea*

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Abstract

Deep-sea corals are the structural foundation of their ecosystems along continental margins worldwide, yet the factors driving their broad distribution are poorly understood. Environmental factors, especially depth-related variables including water mass properties, are thought to considerably affect the realized distribution of deep-sea corals. These factors are governed by local and regional oceanographic conditions that directly influence the dispersal of larvae, and therefore affect the ultimate distribution of adult corals. We used molecular barcoding of mitochondrial and nuclear sequences to identify species of octocorals in the genus *Paramuricea* collected from the Labrador Sea to the Grand Banks of Newfoundland, Canada at depths of 150 to 1500 meters. The results of this study revealed overlapping bathymetric distributions of the *Paramuricea* spp. present off the eastern Canadian coast, including the presence of a few cryptic species previously designated as *Paramuricea placomus*. The distribution of *Paramuricea* species in the western North Atlantic differs from their distribution in the Gulf of Mexico, where five *Paramuricea* species exhibit strong segregation by depth. The different distributional patterns of *Paramuricea* species in these contrasting biogeographic regions provide insight into how water mass structure may shape species distribution. Investigating *Paramuricea* prevalence and distribution in conjunction with oceanographic conditions can help demonstrate the factors that generate and maintain deep-sea biodiversity.

1. Introduction

The significance of deep-sea corals to the structure of their associated communities and the function of the wider bathyal ecosystem has become increasingly recognized in recent years (Roberts et al. 2006). Although much attention has focused on scleractinian corals, octocorals,

47 including sea fans, soft corals, and sea pens, also generate habitat heterogeneity and provide
48 numerous ecosystem services (Watling et al., 2011). These ecosystems of the deep harbor
49 diverse communities, including commercially significant fisheries species (Krieger and Wing,
50 2002; Baillon et al., 2012) and tight symbiotic associations with invertebrates such as crabs,
51 ophiuroids, molluscs, and polychaetes (Krieger and Wing, 2002; Buhl-Mortensen and
52 Mortensen, 2005; Cho and Shank, 2010; Shank, 2010; Girard et al., 2016). Octocorals have been
53 shown to be a potential source for emerging pharmaceuticals (Correa et al., 2011), and have been
54 used as high-resolution paleoclimatic archives (Prouty et al., 2014; Robinson et al., 2014;
55 Sherwood et al., 2014).

56 Despite the increasing awareness of deep-water octocoral ecosystems, various
57 anthropogenic pressures threaten their existence and productivity. In the Gulf of Mexico (GoM),
58 the effects of the Deepwater Horizon oil spill on deep-sea coral communities, primarily
59 structured by *Paramuricea biscaya* Grasshoff 1977, illustrated the potential repercussions of
60 deep-sea oil exploration and extraction to both the immediate and surrounding environment
61 (White et al., 2012; Fisher et al., 2014). The expansion of fisheries activities into deeper waters
62 also poses a threat to deep-sea corals and associated fauna (Watling and Norse, 1998; Fosså et
63 al., 2002; Hall-Spencer et al., 2002), especially to the abundant coral communities in the
64 Canadian North Atlantic where bottom trawling has increased at greater depths (>600 m) since
65 the 1990s (Kulka and Pitcher, 2001; Edinger et al., 2007; Wallace, 2007; NAFO, 2013). Growth
66 rates estimated for many deep-sea octocorals indicate long life spans and slow growth (Andrews
67 et al., 2002; Sherwood and Edinger, 2009), rendering them highly susceptible to anthropogenic
68 disturbances (Neves et al., 2014). For example, radiocarbon (^{14}C) aging for *Paramuricea* spp.
69 collected at depths >1000 m in the GoM revealed *P. biscaya* as old as 168-599 years (Prouty et

al., 2014), whereas two specimens from 814 and 850 m off of Newfoundland and Labrador were dated as 71-103 years old (Sherwood and Edinger, 2009).

The distribution of deep-water octocorals depends on habitat suitability, which is based on key environmental factors including substrate, temperature, salinity, slope, oxygen levels, and productivity (Bryan and Metaxas, 2006, 2007; Yesson et al., 2012). Recent investigations have also revealed that depth-related factors (Long and Baco, 2014; Quattrini et al., 2014; Pante et al., 2015), including water mass (Arantes et al., 2009), bottom geology and geomorphology (Edinger et al., 2011; Baker et al., 2012), and other environmental conditions (Quattrini et al., 2013; Doughty et al., 2014), play varying roles in controlling global and local distribution of octocorals. Despite these recent data, however, information on how environmental parameters influence octocoral distribution at the species level remains scarce. In part, the paucity of studies has been due to the lack of targeted specimen collections coupled with environmental data. With the potential for cryptic, new, and incipient species among deep-sea corals (Pante and Watling, 2012; Bayer et al., 2015) and the difficulties in identifying species from video observations alone, molecular data are necessary to resolve species-specific patterns across various spatial scales (Pante and Watling, 2012; Quattrini et al., 2013). Furthermore, molecular data coupled with environmental data can provide insight into biogeographic patterns of genetic lineages and elucidate potential mechanisms influencing genetic diversity and connectivity within and across regions (Thoma et al., 2009; Quattrini et al., 2013; Pante et al., 2015).

An outstanding question in marine ecology is to what degree oceanographic parameters shape realized patterns of species distributions. Ambient seawater conditions vary depending on the properties of a given water mass, which include temperature, salinity, and density. Water mass structure varies temporally and spatially, and is thus a fluctuating environmental parameter

93 that can affect the distribution of benthic organisms. The relative influence of oceanographic
94 factors on gene flow in marine environments is also dependent on species-specific life-history
95 traits (Neethling et al., 2008), especially an organism's reproductive biology (Nunes et al., 2011).
96 Octocorals not only display varying reproductive modes (e.g. broadcast spawning and brooding),
97 but they also inhabit an impressive depth range and are widely distributed, which demonstrates
98 their capability to survive in a broad range of environmental conditions. Thus, octocorals are
99 well suited to study how oceanographic conditions influence distribution of foundation species in
100 the deep sea.

101 The octocoral genus *Paramuricea* Kölliker, 1865 (Octocorallia: Alcyonacea) has been
102 observed worldwide from the subtidal zone to depths of approximately 2600 m (Sánchez et al.,
103 2003; Thoma et al., 2009; Mokhtar-Jamaï et al., 2011; Baker et al., 2012; Doughty et al., 2014).
104 A recent study in the GoM indicated that at least five *Paramuricea* spp. segregate by depth and
105 are locally abundant on hard bottom, topographic highs (Doughty et al., 2014). Despite these
106 recent ecological genetic studies, the taxonomy of this group remains problematic because
107 morphological characters and genetic barcodes often are not congruent, indicating that additional
108 genetic data are needed to clarify species boundaries (Thoma, 2013). For organisms with
109 widespread distributions such as *Paramuricea* spp., it is critical to resolve species identities to
110 the best of our ability, detect cryptic species, and examine genetic differentiation among
111 populations in order to identify and protect regions that foster larval dispersal and, ultimately,
112 connectivity.

113 The Canadian Atlantic is home to a multitude of deep-sea corals (Wareham and Edinger,
114 2007; Wareham, 2010; Baker et al., 2012). In order to provide adequate ecosystem-based
115 management of deep-sea coral habitats, the Coral and Sponge Conservation Strategy for Eastern

116 Canada has set a target (Target 1, Action 1.2) to increase knowledge of the distribution and
117 abundance of coral species (DFO, 2015). Along the Labrador continental shelf edge and slope,
118 an area containing 14 coral species was documented between Makkovik Bank and Hamilton
119 Bank (Wareham and Edinger, 2007). *Paramuricea* spp. abundantly occur in this region of the
120 Labrador-Newfoundland shelf (Wareham and Edinger, 2007), and approximately half of the
121 specimens in the study were collected from the Makkovik Bank-Hamilton Bank region. Data
122 from widespread fishery observations provide general insight into deep-sea coral communities;
123 however, further taxonomic resolution is necessary to understand biodiversity at the species
124 level. Further, the contrasting oceanography of the western North Atlantic and the GoM presents
125 an opportunity to study the distribution of a widespread genus of deep-sea octocorals with regard
126 to principal water masses.
127 This study examines the genetic diversity and spatial distribution of *Paramuricea* spp. in
128 the western North Atlantic (Labrador Sea to the Grand Banks of Newfoundland) and the GoM
129 with respect to water mass structure. Specifically, we tested whether reduced vertical water
130 column structure leads to overlapping species distributions in the North Atlantic and Labrador
131 Sea, as opposed to the distinct bathymetric breaks in species distributions in the well-stratified
132 GoM. The relationship between the observed bathymetric distributions of genetic lineages and
133 oceanographic regimes reveals the influence of water mass as a mechanism underlying the
134 patterns of distribution, evolutionary history, and biodiversity of deep-sea corals.
135
136 2. Materials and Methods
137 *2.1 Oceanographic background*

The Atlantic Ocean is particularly important to global thermohaline circulation as it is the only ocean basin to feature deep convection at both poles (Oppo and Curry, 2012). In the North Atlantic, this convection generates the North Atlantic Deep Water (NADW), which subsequently affects the world ocean. The NADW is formed by the rapid cooling of the waters of the Gulf Stream as they form the subpolar gyre and mix with the waters of the Labrador Sea (Rhein et al., 2011). The Labrador Sea is characterized by several water masses: 1) the cold Labrador Current along the shelf edge from ~50-200 m, 2) the slightly warmer, modified Irminger Water along the slope ~200-500 m, 3) the Labrador Sea Water (LSW) ranging from ~500 to ~1000-2000 m, depending on the previous winter's convection, 4) the North Atlantic Deep Water at depths of ~2000-3200 m, and 5) the dense bottom-layer Denmark Strait Overflow Water >3000 m (Lazier et al., 2001, 2002; Khatiwala et al., 2002; Yashayaev, 2007). Wintertime deep convections in the Labrador Sea form the well-mixed LSW that circulates in the western North Atlantic and beyond. This winter convective activity has been documented to depths of 2300 m (Dickson et al., 1996).

In contrast to the homogeneity of the principal water mass (LSW) in the Labrador Sea, the GoM is composed of water mass layers of distinct depth ranges. The deep-water of the GoM is only ventilated from the Caribbean above the Yucatan Sill (2040 m) by the NADW (Rivas et al., 2005). The principal water masses in the GoM include: 1) the Subtropical Underwater from <200-250 m, 2) the Sargasso Sea Water between 200-400 m, 3) the Tropical Atlantic Central Water from 400-600 m, 4) the Antarctic Intermediate Water at depths of approximately 600-1000 m, and 5) the NADW at depths >1000 m (Nowlin et al., 2001; Rivas et al., 2005). These depths vary seasonally and east-to-west across the GoM depending on the structure of the Loop Current and the westward propagating eddies that it sheds.

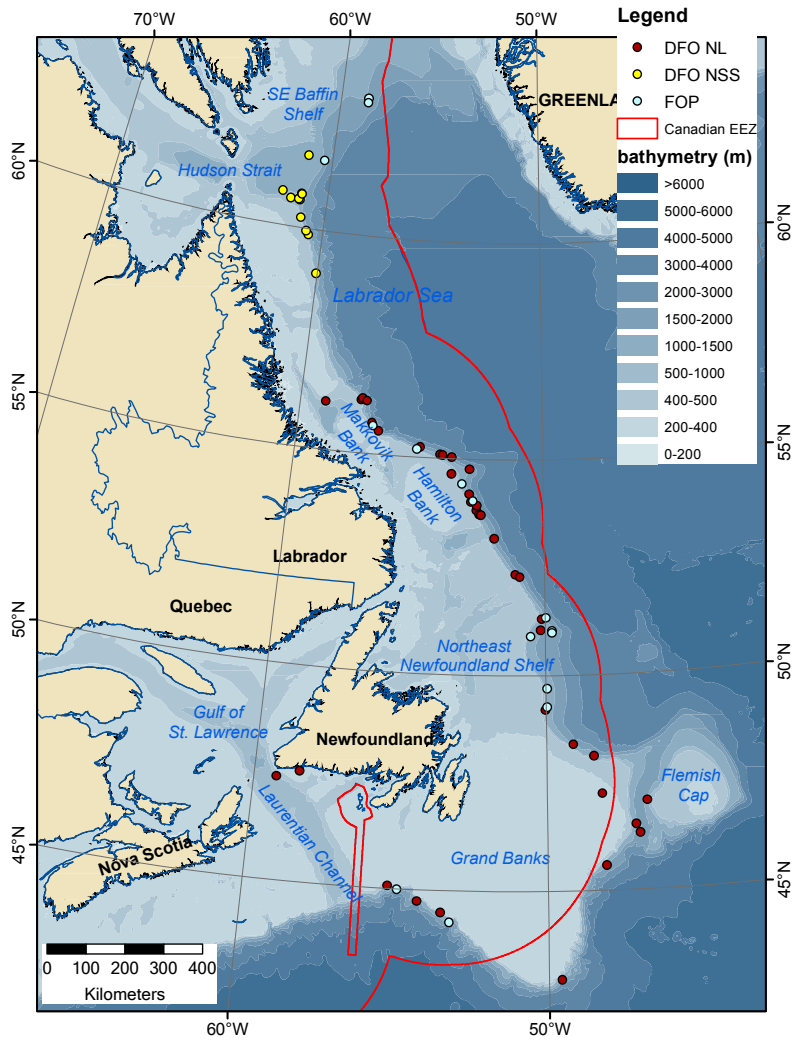

Figure 1. *Paramuricea* specimens collected between 2005 and 2011 in the Northwest Atlantic, from the Southeast Baffin Island slope down to the Grand Banks of Newfoundland. Corals were acquired by the Canadian Department of Fisheries and Oceans (DFO) of Newfoundland and Labrador (NL) during collaborative surveys between the DFO and the Northern Shrimp Industry (NSS), and by the Fisheries Observer Program (FOP).

2.2 Sample Collection

Newfoundland and Labrador samples (Fig. 1): Samples were opportunistically collected as fishery by-catch between 2005 and 2011 during Canadian Department of Fisheries and Ocean (DFO) multi-species stock assessment trawl surveys, DFO-sponsored Northern Shrimp Stock

Assessment (NSS) trawl surveys, and by the Fisheries Observer Program (FOP) on commercial fishing vessels using several gear types including trawl, bottom longline, or bottom gillnet. Specimens in this study were collected from the Hudson Strait to the Grand Banks of Newfoundland at depths of 150-1500 m, with most samples collected from the continental slope. DFO multi-species surveys and the NSS surveys follow depth-stratified random design using a Campellen 1500 shrimp trawl, towing along contour for approximately constant depth for each trawl tow. Using SCANMAR acoustic trawl instrumentation, mean depth for each tow was derived for the duration of each 15 minute (approximately 0.8 nautical mile) tow, starting from initial bottom contact through the end of the tow (McCallum and Walsh, 1996). Sample depths from the DFO (n=33) and NSS (n=6) ranged from 152-1494 m and 452-617 m, respectively, while the FOP samples (n=13) ranged from 457-1222 m depth. Total sampling effort in the DFO trawl surveys during this period was 7958 tows, with 0.4% containing *Paramuricea* samples, while 0.5% of the 1305 NSS tows yielded *Paramuricea* samples. Total effort for the FOP program is not available. Specimens were frozen at -20°C and then preserved in 100% ethanol.

Gulf of Mexico samples (Fig. 2): Specimens were collected from 21 sites in the northern GoM and two sites along the West Florida Slope during seven cruises in 2009-2011 using remotely operated vehicles (ROVs *Jason, Seaview, Mohican, Schilling UHD)* and human-occupied vehicles (HOVs *Alvin* and *Johnson-Sea-Link*). Tissue samples (2-3 cm) were frozen at -80ºC and preserved in both 95% ethanol (stored at -20 ºC) and a high-salt EDTA preservative (stored at -80 ºC). Subsamples were obtained from each voucher specimen and either preserved in 95% ethanol or dried (further sampling details can be found in Doughty et al., 2014; Quattrini et al., 2014).

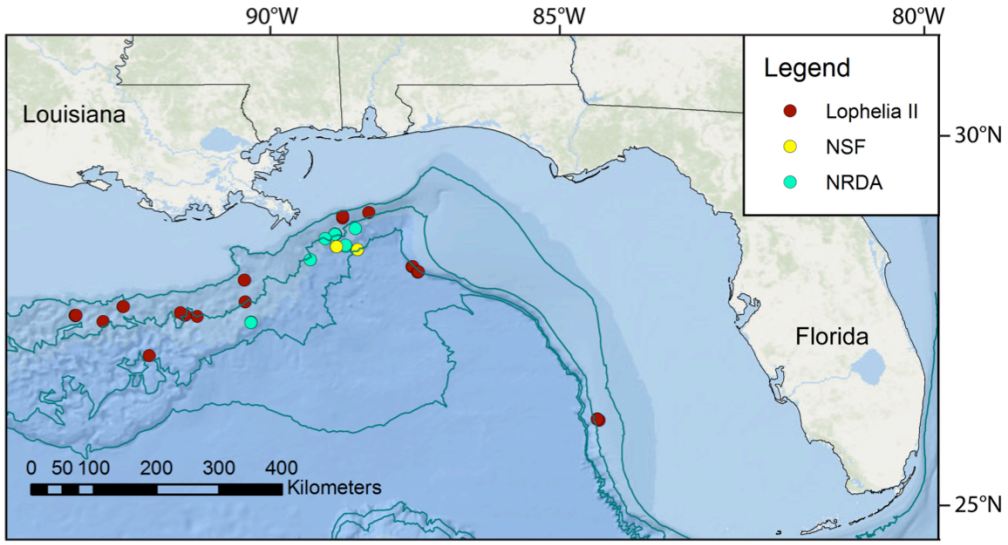

Figure 2. *Paramuricea* specimens collected between 2009 and 2011 in the Gulf of Mexico. Corals were acquired during Lophelia II, NSF, and NRDA scientific cruises. Bathymetry contour lines of 200 m, 1000 m, 2000 m and 3000 m are displayed (Source: USGS and CMGP).

2.3 Molecular Barcoding

Total DNA was extracted from Canadian specimens (n=52) using the Qiagen DNeasy Blood and Tissue kit. Following McFadden et al. (2011), an extended mitochondrial barcode, including the cytochrome oxidase I region (*cox1*) with an adjacent intergenic region (*igr1*) and the octocoral-specific mismatch recognition protein (*mtMutS*), were sequenced (GENEWIZ and GenScript) for Canadian samples (n=51). We also added the available mitochondrial sequences for *Paramuricea* (n=109) that were previously published (Doughty et al., 2014; Quattrini et al., 2014). These data consisted of mitochondrial genetic types A, B3, E, and H, as well as *P. biscaya*, which currently consists of three mitochondrial types: B1, B1a, and B2 (Table S3).

In addition to the mitochondrial data, the 28S nuclear ribosomal gene was also sequenced for both Canadian samples (n=45) and samples from the Gulf of Mexico (n=94). Fragment sizes of approximately 850 bp from the 5′ end of the *mtMutS* gene (ND42599F and Mut3458R), 800 bp of the *cox1+igr1* region (COII8068F and COIOCTR), and 800 bp of the 28S rDNA gene

214 (28S-Far, 28S-Rar, and 28S-Rab) were PCR amplified according to published protocols (France
215 and Hoover, 2002; McFadden et al., 2004, 2011; McFadden and van Ofwegen, 2013; Quattrini et
216 al., 2014).

217 The resulting nucleotide sequences were edited, aligned by ClustalW (gap opening
218 penalty= 20, extension penalty= 15) and visually adjusted by viewing amino acid alignments in
219 MEGA v5 (Tamura et al., 2011). After quality trimming, sizes of the gene regions were as
220 follows: 736 bp for *mtMutS,* 692 bp for *cox1+igr1* and 625 bp for 28S. The program PHASE v
221 2.1 (Stephens et al., 2001; Stephens and Scheet, 2005) was used to resolve alleles of 28S rDNA.
222 Uncorrected p-distances were calculated at the mitochondrial loci (1428 bp alignment) and
223 nuclear loci (625 bp alignment) and across the entire alignment (2053 bp alignment, including
224 gaps) (MEGA v5; Tamura et al. 2011). Since species boundaries remain unclear in this genus,
225 we coded genetic types based on differences at each sequenced region: uppercase letters denote
226 different *mtMutS* sequences, numbers denote different *cox1* genetic types*,* lowercase letters
227 designate *igr1* sequences (if different), and numbers following a dash indicate different 28S
228 rDNA sequences.

229 Phylogenetic parsimony networks were created for the mitochondrial dataset and for the
230 nuclear 28S rDNA using the program TCS v 1.2.1 (Clement et al., 2000). All phased alleles for
231 heterozygote colonies were included in the 28S network. Both Gulf of Mexico and Canadian
232 samples were included in these analyses.

233 Bayesian analysis was performed separately on mitochondrial data *(mtMutS+cox1+igr1*)
234 and on the 28S data to examine phylogenetic relationships within the genus *Paramuricea* in the
235 North Atlantic. This analysis included each unique genotype found in each region and sequences
236 available on GenBank (NCBI) that had accompanying published data, including: Plexauridae,

237 *Paramuricea placomus, Paramuricea biscaya*, *Paramuricea multispina, Paramuricea* n. sp.,
238 unidentified *Paramuricea* spp. (haplotypes A, C, D, G, F), and *Placogorgia* spp. (Table S3). 28S
239 data was available for only one Plexauridae and one *Placogorgia* sp. Data were partitioned by
240 gene region and the following substitution models were applied: GTR+G to *mtMutS*, GTR was
241 applied to *igr1* and HKY+G was applied to *cox1* (AIC, JModeltest v 0.1; Posada, 2008). GTR
242 was also applied to the 28S data (AIC, JModeltest v 0.1). Convergence was assessed using both
243 the Estimated Sample Size (ESS >2000) and Potential Scale Reduction Factors (PSRF=1.0).
244 Unique *mtMutS* haplotypes were overlaid onto the tips of tree. The number of generations was
245 set to 5,000,000 with a sampling frequency of every 1,000 generations and a burnin of 10,000
246 trees. The consensus trees were rooted with *Acanthogorgia* spp. and drawn in FigTree (v 1.4,
247 http://tree.bio.ed.ac.uk/software/figtree/). Citation, depth, and location information of species
248 used in analyses with corresponding GenBank numbers are listed in Table S3.
249
250 3. Results
251 *3.1 Genetic Analyses*
252 The concatenated mitochondrial and nuclear data (2053 bp, *mtMutS*+*cox1*+*igr1*+28S)
253 resulted in 18 genotypes (including four heterozygotes) among both Canadian (n=52) and Gulf
254 of Mexico (n=94) samples (GenBank accession numbers: KX267322-KX267562; Table S1).
255 Only two of the genotypes (*Paramuricea* type A4-2 and A4-3) occurring off Canada were also
256 found in the GoM (Fig. 3, Table S2). Seven unique genotypes were present off Canada and nine
257 unique genotypes occurred only in the GoM (Figs. 3 and 4, Table S2). The most common
258 genotype among the Canadian samples was *Paramuricea* type A4-1 (n=30), followed by
259 *Paramuricea* type C6c-1 (n=3) and type D5-1 (n=3). Other genotypes from Canada had either

one or two colonies. In the GoM, the most common type was *Paramuricea* type B3-6 (n=28), followed by type B1-6 (n=25,) and type B2-6 (n=19).

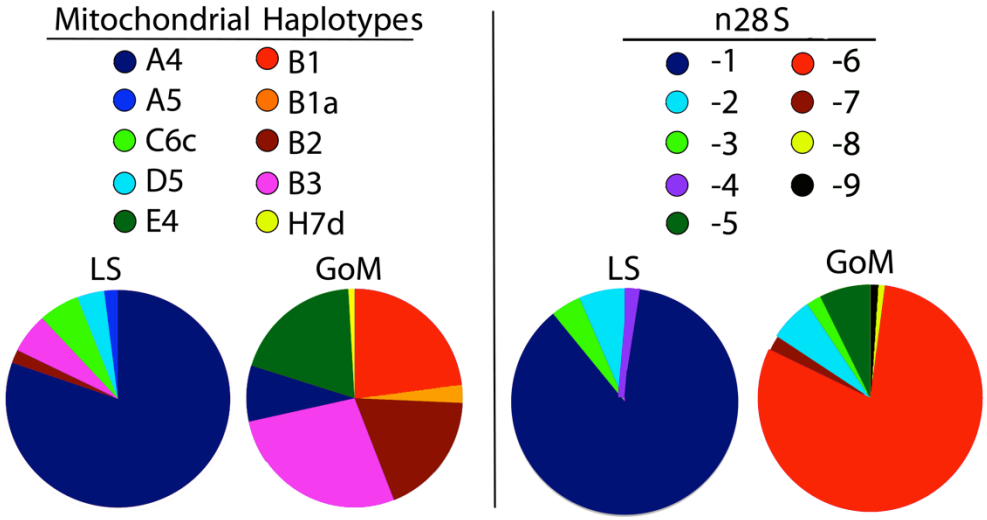

Figure 3. Mitochondrial (*mtMutS+igr1+cox1*) and nuclear (28S) alleles of *Paramuricea* from the Labrador Sea (LS) region and the Gulf of Mexico (GoM).

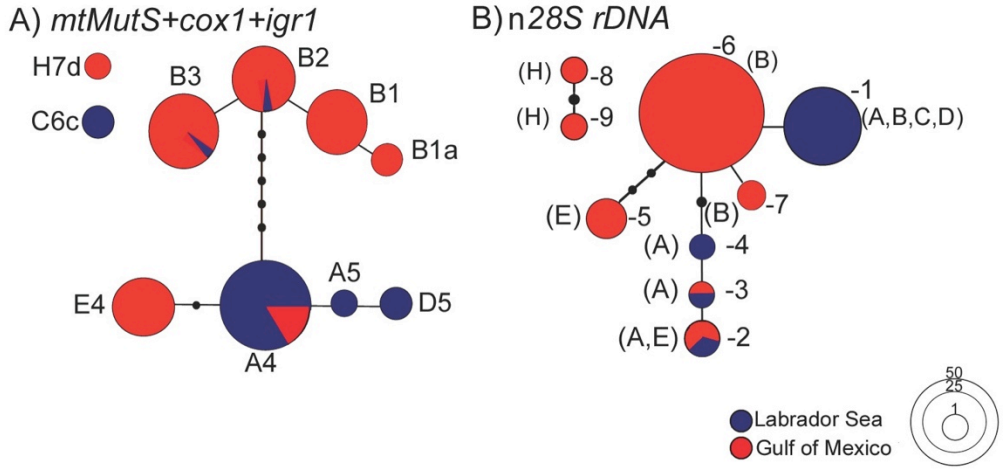

Figure 4. Phylogenetic parsimony networks of *Paramuricea* genetic types at A) an extended mitochondrial *mtMutS+cox1+igr1* barcode and B) nuclear 28S rDNA. The network of nuclear 28S sequences includes phased sequences for heterozygote colonies. For nuclear 28S sequences in (B), corresponding mitochondrial haplotypes are indicated in parentheses. Lines connecting alleles denote one mutational step; small black circles represent possible, but not sampled, alleles. If no lines connect alleles, then datasets represent unconnected subnetworks. Circle size corresponds to number of colonies sequenced for the particular genetic type. Colors denote

samples collected in the Labrador Sea (blue) and in the Gulf of Mexico (red). Those with black squares are from GenBank (see Table S3).

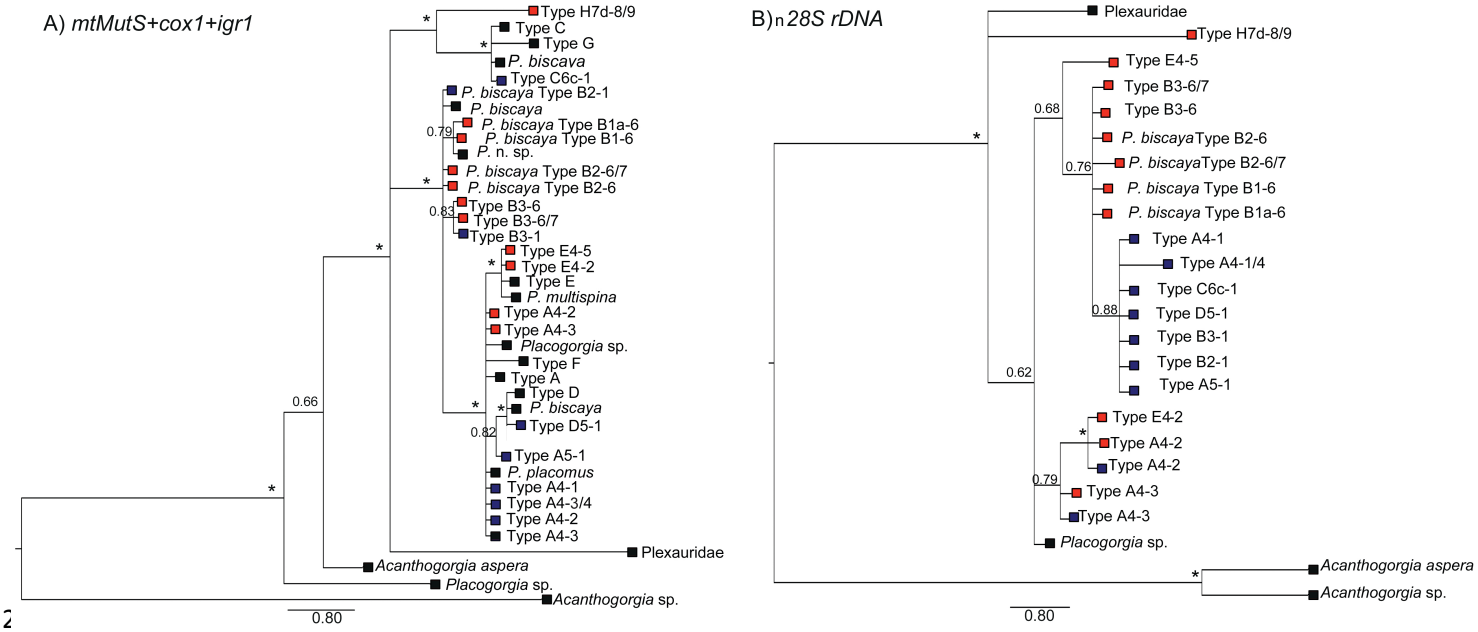

Figure 5. Bayesian inference of *Paramuricea* from the western North Atlantic with additional GenBank sequences (Supplementary Information Tables S1 and S3). Phylogeny of A) the mitochondrial extended barcode (*mtMutS+cox1+igr1*) and B) nuclear 28S rDNA. Species and mitochondrial *mtMutS+cox1+igr1* genetic types are color-coded (blue= Labrador Sea; red= Gulf of Mexico). Posterior probabilities are >0.95 unless indicated.

Phylogenetic network analysis and Bayesian analysis revealed incongruence in mitochondrial and nuclear data, particularly among colonies collected off Canada (Figs. 4 and 5). Specimens from the Labrador Sea had different mitochondrial haplotypes, but the nuclear 28S-1 sequence was the same across these haplotypes (A, B, C, D). In contrast, the majority of colonies in the GoM with unique mitochondrial haplotypes had unique nuclear sequences. An exception to this pattern was that *Paramuricea* type B haplotypes shared the same nuclear 28S-6 sequence. Phylogenetic network analysis also revealed two unconnected subnetworks for both *mtMutS* and 28S (Fig. 4). Results from the Bayesian analyses indicated incongruence in morphological

identifications and genetic data, particularly in regard to the placement of specimens that were previously identified as *P. biscaya* in multiple clades (Fig. 5).

Genetic distances (p-distance) were slightly higher at the nuclear 28S locus compared with mitochondrial haplotypes. Mitochondrial haplotypes were 0.07 to 2.38% divergent, whereas nuclear 28S alleles were 0.16 to 2.88% divergent. Six mitochondrial haplotypes were present off Canada and seven were found in the GoM (Fig. 3, Table S2). However, only four 28S alleles (28S-1 to -4) were present in the Canadian *Paramuricea* samples (p-distance 0.16-0.80%), compared to seven 28S sequences (28S-2, 3, 5-9) identified in GoM samples (p-distance 0.16-2.88%; Table S2). Notably, the most common nuclear sequence (28S-1) in specimens collected off Canada was present in 41 colonies with six different corresponding mitochondrial haplotypes (A4, A5, B2, B3, C, and D). The other three 28S sequences were only present in the abundant mitochondrial type A4 corals in Canada (Fig. 4B, Table S2). In contrast, a unique 28S sequence occurred with each of the seven mitochondrial haplotypes in the GoM. Only two of the same 28S sequences (types 28S-2 and -3) were found in the GoM and off Canada whereas three mitochondrial types (A4, B2, and B3) were found in both regions (Figs. 3 and 4). One heterozygous 28S sequence was recovered from a Canadian sample, with a single nucleotide polymorphism (SNP) at position #74 in the 28S sequence (corresponding to sequences 28S-3 and 28S-4 in Fig. 4B). Three heterozygotes occurred in the GoM samples. One B2 colony and one B3 colony each had a SNP at position #522 in the 28S sequence (corresponding to sequences 28S-6 and 28S-7, Fig. 4B). *Paramuricea* type H had SNPs at position #332, 350, and 356 (denoted by 28S-8 and 28S-9).

For concatenated mitochondrial and nuclear data, p-distances ranged from 0.05-2.44% among different colonies (Table S2). *Paramuricea* type C collected off Canada and *Paramuricea*

type H collected in the GoM were the most genetically divergent from the rest of the *Paramuricea* colonies (Figs. 4 and 5, Table S2). P-distances were 0.93-1.41% between *Paramuricea* type C colonies and others and 1.80-2.44% between *Paramuricea* type H and other colonies. Concatenated sequences of colonies in the *Paramuricea* type B group (as designated by *mtMutS*) were divergent (0.29-0.68% p-distance) from the groups A, D, and E (Fig. 4B, Table S2). Variants in the *Paramuricea* B group (B1-6, B1a-6, B2-6, B2-6/7, B3-6, and B3-6/7) were highly similar (0.00-0.19% p-distance) (Fig. 5, Table S2). Additionally, little divergence (0.05-0.24% p-distance) occurred among genetic type A variants (A4-1, A4-2, A4-3, A4-3/4, and A5-1) and *Paramuricea* type E variants (E4-2, E4-5) (0.24% p-distance). P-distances between *Paramuricea* groups A and D off Canada and in the GoM and E in the GoM ranged from 0.05-0.44% (Table S2).

3.2 Bathymetric Distributions

Paramuricea genotypes from Labrador and Newfoundland appear to be distributed throughout most of the depth range sampled (Fig. 6). The most abundant genetic type, *Paramuricea* type A4, was present throughout the sampled depth range from 152-1494 m. *Paramuricea* type A5 was collected at a depth of 936 m and *Paramuricea* type B2 at 827 m, while *Paramuricea* type B3 was discovered at 556 and 1245 m. *Paramuricea* type C had a range of 609-1182 m and *Paramuricea* type D had a distribution of 617-1086 m in this study. The most common nuclear 28S-1 sequence was distributed throughout the water column at depths of 152-1494 m across all mitochondrial types (A4, A5, B2, B3, C, D) from Canada. The other nuclear 28S sequences (types 28S-2, 28S-3, and 28S-3/4) were all recovered from *Paramuricea* type A4 colonies <680 m.

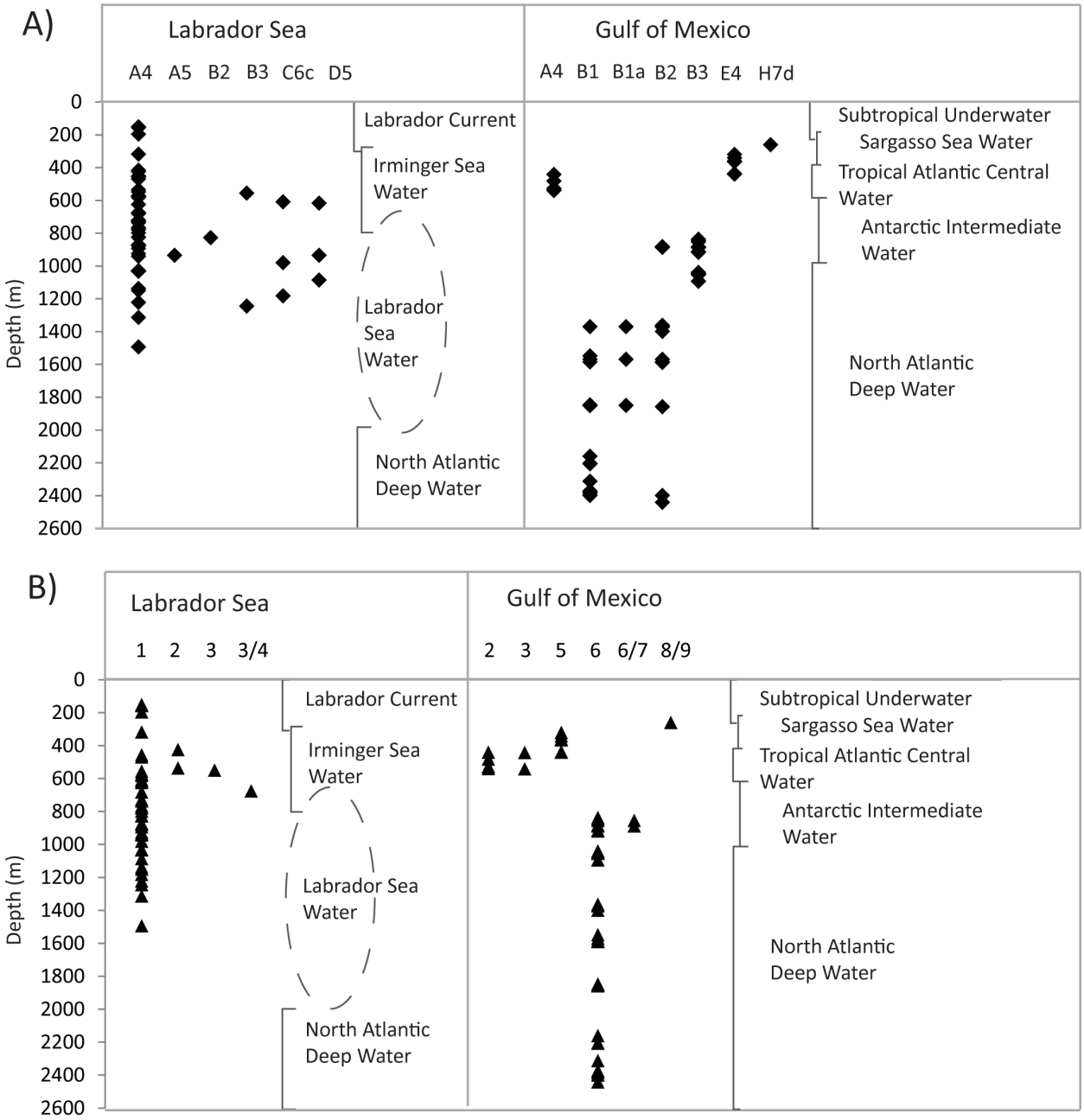

Figure 6. Distribution of *Paramuricea* A) mitochondrial (*mtMutS+cox1+igr1*) haplotypes and B) nuclear 28S sequences in the Labrador Sea and Newfoundland, Canada compared to the Gulf of Mexico (Doughty et al., 2014). Principal water mass layers are indicated for each geographic location. Triangular markers for the Gulf of Mexico represent multiple coral specimens (Doughty et al., 2014), while each marker for *Paramuricea* spp. in the Labrador Sea and Newfoundland represents one coral specimen.

In the GoM, nuclear sequence 28S-6 (from all *Paramuricea* type B variants B1, B1a, B2, and B3) was found over a wide depth range of 845-2441 m, with heterozygote 28S-6/7 (from *Paramuricea* B2 and B3) recovered from 854 and 888 m. The remaining five 28S sequences were recovered from a narrower depth range <541 m. Specifically, type 28S-2 (from *Paramuricea* types A and E) was recovered from 441-549 m, and type 28S-3 (from *Paramuricea* type A) from 443 and 541 m. Type 28S-5 (from *Paramuricea* type E4) was recovered from 320-441 m, and the heterozygote 28S-8/9 (from *Paramuricea* type H) was collected at the shallowest depth (260 m) sampled.

4. Discussion

Despite the notion of open oceans, depth-associated factors may segregate species and contribute to (pre-zygotic) isolation in marine environments (Prada and Hellberg, 2013). Even at scales of several hundred meters, the presence of distinct water mass layers affects the distribution of deep-water coral species (Long & Baco 2014) and populations (Quattrini et al., 2015). Our study examines the influence of water mass structure on the species-level distribution of the octocoral *Paramuricea* over depth ranges of 1000 and 2000 m for the Labrador Sea and Gulf of Mexico, respectively. The transitions between water masses, which are characterized by distinct physical and chemical properties, can function as both conduits for larval dispersal and also as intrinsic barriers to the vertical transport of coral larvae, which ultimately could limit gene flow. The properties and stratification of water mass layers may also contribute to reproductive isolating mechanisms as larvae cannot tolerate environmental changes that co-occur with water masses, subsequently leading to immigrant inviability (Nosil et al., 2005). Our study reports the overlapping distribution of *Paramuricea* spp. within the well-mixed LSW mass in

contrast to the segregation observed in the stratified waters of the Gulf of Mexico. Comparing *Paramuricea* species distribution between geographic locations under different oceanographic regimes provides insight into the environmental factors that influence speciation in the deep sea.

4.1 Distribution Patterns in the Labrador Sea

The Labrador Sea is a particularly interesting location to study temporal variations of convections and ocean currents, which vary greatly and have far-reaching effects on oceanographic patterns (Yashayaev, 2007). Wintertime heat loss from the Labrador Sea primes the region for deep ocean convection, and subsurface float measurements have revealed mixed layers from 400-1300 m in the Labrador Sea (Lavender et al., 2002), indicating the homogeneity of the LSW. However, due to the influence of the North Atlantic Oscillation, the maximum depth of convection in the Labrador Sea varies (up to 2300 m) in response to substantial interannual and interdecadal ocean-atmosphere differences (Dickson et al., 1996). Previous data recorded convection depths of less than 700 m in 2007, but in the following year convections reached 1600 m (Yashayaev and Loder, 2009). By creating a broad homogeneous water mass, the deep convections forming the LSW appear to influence the wide bathymetric distribution of *Paramuricea* species over the sampled depth range in the Labrador Sea region.

The homogeneity of the LSW may affect the distribution of deep-sea corals in this region due to the stability of this large water mass. Between 1960 and 2005, the LSW was characterized by temperatures of 2.7-3.7°C and salinity of ~34.83-34.91 (Yashayaev, 2007). The frequency and extent of LSW mixing and its narrow temperature regime present a more consistent environment over a large depth range in which coral larvae could disperse. Vertical transport of larvae could be accomplished by the deep winter convection that mixes the water column, supporting the distribution of genotypes across a bathymetric range of nearly 1500 m. Except for

393 *Paramuricea* type A4-2, all other genetic types off Canada were recovered from 550 to >1000 m,
394 and were not depth-stratified within the study area. Notably, *Paramuricea* type A4-1,
395 representing *P. placomus,* was found throughout the surveyed depth range (150-1500 m) and
396 *Paramuricea* type B3 was recovered from 556 and 1245 m. The mixed LSW thus does not
397 appear to present a conspicuous barrier to dispersal over the 150-1500 m bathymetric range.
398 Additional surveys of the NADW mass off Canada and further south would corroborate the role
399 of water masses on species distribution.

400 *4.2 Comparison with the GoM*

401 In contrast to our findings that *Paramuricea* species (sampled from 550-1250 m) are
402 evenly distributed over depth in the Labrador Sea, previous research in the GoM demonstrated
403 that *Paramuricea* mitochondrial haplotypes are segregated by depth (Doughty et al., 2014) (Fig.
404 6). Distinct water mass layers in the GoM may limit gene flow by impeding the transport of
405 larvae among the stratified water layers. However, a recent study documented that deep ocean
406 current boundaries in the North Atlantic do not physically impede larval dispersal (Etter and
407 Bower, 2015). Therefore, population divergence across water masses boundaries in the GoM
408 may be driven by immigrant inviability and strong, pre-reproductive selection that promotes
409 adaptive divergence (Prada and Hellberg, 2013; Quattrini et al., 2015).

410 There appears to be a correlation between the water mass layers in the GoM and the
411 distribution of *Paramuricea* species. Doughty et al. (2014) found that the five species of
412 *Paramuricea* in the GoM are segregated by depth, and our data shows that these depth
413 distributions generally correspond to water mass layers present in the GoM. *Paramuricea* type H
414 specimens were recovered from the shallowest depth (259 m) and type E4 was found exclusively
415 at sites <441 meters deep, coinciding with the Sargasso Sea Water layer that lies between

approximately 200-400 m. *Paramuricea* type A4 was identified from collections between 443-541 m, corresponding to the Tropical Atlantic Central Water mass found at depths of 400-600 m. *Paramuricea* type B3 was recovered at depths of 835-1090 m, which coincides with the Antarctic Intermediate Water typically found in the GoM at 600-1000 m. Finally, *P. biscaya* (types B1, B1a, and B2) was identified at the deepest depths sampled in the GoM between 1370-2600 m (with three colonies from ~850-900 m), at which the NADW is observed (>1000 m). *Paramuricea biscaya* has been collected from seamounts in the western North Atlantic at depths of 1200-2200 m (Thoma, 2013) and in the Bay of Biscay at 2000 m (Grasshoff 1977). Off eastern Canada, only one type B2 colony (likely *P. biscaya*) was collected among the Labrador Sea samples and it was found at a mean depth of 827 m.

Table 1. Average temperature and salinity by depth of sampling regions in the Labrador Sea (44-64°N 46-62°W) and the Gulf of Mexico (29-27°N 87-94°W). High resolution CTD data was accessed through the World Ocean Database (Boyer et al., 2013). *The depth of the Labrador Sea Water mass varies annually.

Labrador Sea				**Gulf of Mexico**		
Temp.	Salinity		Depth (m)		Temp.	Salinity
1.10°C	33.07	Labrador Current	50-199	Subtropical Underwater	18.77°C	36.27
3.64°C	34.38	Modified Irminger Water	200-399	Sargasso Sea Water	12.21°C	35.54
3.75°C	34.85	Labrador Sea Water*	400-599	Tropical Atlantic Central Water	8.52°C	35.04
			600-999	Antarctic Intermediate Water	5.96°C	34.91
			1000-1999	North Atlantic Deep Water	4.37°C	34.97
2.80°C	34.90	North Atlantic Deep Water	2000-2999			
2.04°C	34.90	Denmark Strait Overflow Water	>3000			

The differences in temperature and salinity of the water masses create distinct environments that may offer optimal conditions for certain species (Table 1). Such abiotic factors

435 may have considerable influence on limiting gene flow, initial larval settlement, and on the
436 physiology of adult colonies. In the GoM, however, dissolved oxygen had a weak correlation
437 with octocoral (*Callogorgia*) distribution over depth due to the overlapping species distribution
438 over dissolved oxygen gradients (Quattrini et al., 2013). Different environmental conditions may
439 lead to immigrant inviability and ultimately adaptive divergence, especially for organisms that
440 take years to decades to reach full reproductive maturity (Prada and Hellberg, 2013).

441 Environmental gradients related to depth can affect species distribution and lead to
442 ecological niche segregation. Coral assemblages on continental shelves and slopes can be linked
443 to differences in substrate availability and preference within the lower/mid versus upper slope
444 (Arantes et al., 2009). However, depth-related variables may exert stronger influences on deep-
445 sea octocoral assemblages, with depth strongly indicative of octocoral population structure over
446 wide spatial scales (Pante et al., 2015; Quattrini et al., 2015). Environmental factors, including
447 local oceanographic conditions, must be closely examined in conjunction with species
448 boundaries. In the Mediterranean Sea, regional thermohaline differences and the range of
449 seasonal thermoclines are thought to contribute to the genetic differentiation of *Paramuricea*
450 *clavata* (Mokhtar-Jamaï et al., 2011). Octocoral assemblages in the western South Atlantic were
451 also related to water mass structure, with significant differences between upper (≤760 m) and
452 mid/lower (1000-1605 m) slope communities and more homogeneous assemblages across the
453 lower slope, influenced by the NADW, compared to the mid slope (Arantes et al., 2009). While
454 oceanographic barriers to dispersal are obviously significant, the ecology and biotic interactions
455 among species may also influence realized species distributions.

456 *4.3 Incongruent Patterns among Molecular Data*

457 Discordant patterns between the mitochondrial and nuclear loci were evident among
458 colonies, but the level of this discordance depended on geographical region. Off Canada, the
459 nuclear 28S-1 sequence was found in six different mitochondrial haplotypes (A4, A5, B2, B3,
460 C6c, D5) spanning a broad depth range (152-1494 m). Only three other unique nuclear 28S
461 sequences (28S-2, 28S-3, 28S-4) were amplified in colonies off Canada, and these were all from
462 the common *Paramuricea* A4 haplotype. In contrast, 28S sequences were generally congruent
463 among colonies with differing mitochondrial haplotypes in the GoM, with two exceptions. The
464 sequence 28S-2 was common in *Paramuricea* A4 colonies, but was also found in one colony
465 with mitochondrial haplotype E4. All four mitochondrial haplotype B variants (B1, B1a, B2, and
466 B3) had the same 28S-6 sequences, which were found throughout a broad depth range (884-2399
467 m) corresponding to the NADW.
468 Mito-nuclear discordance is common in recently diverged taxa (Toews and Brelsford,
469 2012). Smaller effective population sizes of mitochondrial genomes generally allow for complete
470 lineage sorting of the mitochondrial DNA compared with nuclear DNA, the latter of which can
471 often contain ancestral alleles (Funk and Omland, 2003). However, the slow mitochondrial
472 mutation rates in octocorals, owing to a unique mitochondrial mis-match repair gene (*mtMutS)*
473 (Shearer et al., 2002; Bilewitch and Degnan, 2011), could potentially cause incomplete lineage
474 sorting in the mitochondrial genomes of octocorals. This scenario is likely for *Paramuricea* type
475 B variants, as Doughty et al. (2014) noted that *Paramuricea* type B3 was morphologically quite
476 different from B1 and B2. Recent studies using RADseq methods have noted that different
477 species of octocorals can share the same *mtMutS* haplotype (Pante et al., 2015), although this is
478 believed to be fairly rare in octocorals. Because the level of incongruence between mitochondrial
479 and nuclear data in *Paramuricea* spp. differed between biogeographical regions, it suggests that

480 additional factors beyond incomplete lineage sorting may be contributing to this discordance
481 (Funk and Omland, 2003; Toews and Brelsford, 2012).
482 The presence of one nuclear 28S sequence (28S-1) amplified in colonies off Canada with
483 different mitochondrial haplotypes could be driven by hybridization through sex-biased
484 dispersal. Although the reproductive strategy for deep-sea *Paramuricea* spp. is unknown, in the
485 congener *P. clavata*, eggs are fertilized on the mother colony (Coma et al., 1995). Thus, female
486 *Paramuricea* likely have reduced dispersal rates compared with males, leading to genetic
487 structuring in the mitochondrial genomes while male-biased dispersal could maintain gene flow
488 in nuclear DNA (López-Uribe et al., 2014). Sperm-mediated gene flow has also been suggested
489 to influence mito-nuclear discordance in a Caribbean sponge (DeBiasse et al., 2014). Because the
490 homogenous LSW mass supports extensive mixing of the water column, there is a greater
491 potential for gamete dispersal by males and hybridization between species. Also, in the deep
492 GoM (>1000 m) where the homogenous NADW occurs, the mitochondrial B type variants (B1,
493 B1a, B2, B3) all had the same nuclear sequence. It is possible that broad dispersal of sperm from
494 all of these mitochondrial types is leading to hybridization between these recently diverged
495 lineages.
496 Hybridization would be more likely if the species had not had sufficient time to diverge,
497 which could result from long life spans and long times to first reproduction. Although
498 information regarding sexual maturity in *Paramuricea* spp. is limited, it is estimated that *P.*
499 *clavata*, a surface brooder, matures upon reaching a size of 20 cm at an average of 13 years of
500 age (Coma et al., 1995). The slower growth rate of *Paramuricea* spp. in the deep-sea (Prouty et
501 al., 2014) likely makes the time to first reproduction significantly longer. Based on the growth
502 rates published in Prouty et al. (2014), and assuming a size of 20 cm for first reproduction, *P.*

503 *biscaya* would be between approximately 90 and 600 years of age at first reproduction
504 (excluding a single outlier that would be less than 2 years of age). These long generation times
505 would promote slow divergence rates, and increase the probability of hybridization. It remains to
506 be empirically tested whether this is a common contributor to deep-sea coral hybridization rates.
507 It is clear that more nuclear loci are necessary to resolve species boundaries and
508 disentangle the prevalence of introgression and/or incomplete lineage sorting among recently
509 diverged lineages of *Paramuricea*. Octocoral lineages that are >0.5% divergent (p-distance) are
510 likely separate species (McFadden et al., 2011), but in our study genetic distances between
511 putative *Paramuricea* spp. were even lower than this suggested limit. For example, *Paramuricea*
512 cf. *placomus,* represented by A4-1 off Canada and A4-2/3 in the GoM, is morphologically
513 distinct (Grasshoff 1977) from *P. biscaya* (represented by B1, B1a, and B2 in the GoM). Genetic
514 distances based on concatenated data between these two species ranged from 0.29-0.58% (0.42-
515 0.56% mitochondrial data only, Table S2), suggesting that lower p-distances can also be
516 indicative of distinct species. It is possible for a single species to have more than one *mtMutS*
517 haplotype, but evidence also exists for unrecognized cryptic species in cases where there is more
518 than one *mtMutS* haplotype per putative morphospecies (McFadden et al., 2011). Nevertheless,
519 *mtMutS+cox1* provides an important initial evaluation when identifying octocoral species and
520 given the low diversity and variation in 28S sequences, the possible occurrence of 28S paralogs
521 are not likely to affect the conclusions. In another deep-water octocoral genus, *Chrysogorgia,*
522 thousands of SNP loci recovered from RADseq analyses were shown to generally be congruent
523 with the *mtMutS* phylogeny (Pante et al., 2015). Morphological examination coupled with
524 RADseq data would be useful to further resolve genetic and morphological incongruences within
525 the genus, while elucidating evolutionary processes important in homogenizing or increasing

genetic diversity within *Paramuricea.* Our data support that *P. placomus* off Canada inhabits a wide depth range in a homogenous water mass whereas *Paramuricea* spp. in the GoM segregate by depth corresponding to water mass boundaries.

5. Conclusions

Given the uncertain potential for recovery of deep-sea corals due to their slow growth and vulnerability to anthropogenic impacts, conservation efforts are urgently needed to protect these fragile ecosystems. However, it is impossible to manage a resource if basic biological information, such as accurate species identifications and distributions, are lacking. As a widely distributed deep-sea coral genus, *Paramuricea* is an integral part of deep-water marine ecosystems and is currently highly threatened by bottom trawling, oil extraction, and global ocean change. The distribution of *Paramuricea* species in the Labrador Sea and off Newfoundland appears to be influenced by the homogenous Labrador Sea Water as genotypes were distributed throughout this water mass over a large depth gradient. The result is in contrast to the distribution of *Paramuricea* in the Gulf of Mexico, where species appear to be partitioned into depth ranges that correspond to distinct water mass layers. These differences in regional distribution relative to oceanographic conditions are important to consider when examining genetic connectivity, larval dispersal, and habitat niches. The influence of oceanographic conditions on species distributions thus varies from location to location, and must be considered in conservation and management planning. The diversity of previously unidentified, and still undescribed, *Paramuricea* spp. found in localities with enhanced biodiversity in the western North Atlantic require further study and garner further support for the protection of these areas.

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