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Deep-sea octocorals and antipatharians show no evidence of seamount-scale endemism in the NW Atlantic

Jana N. Thoma*, Eric Pante*, Mercer R. Brugler, Scott C. France**

University of Louisiana at Lafayette, Department of Biology, PO Box 42451, Lafayette, Louisiana 70504, USA

ABSTRACT: Seamounts are undersea mountains commonly characterized by accelerated currents, exposed hard-substrates, and relatively high biomass and biodiversity. Hydrographic features associated with seamounts have led authors to hypothesize that benthic invertebrate populations from geographically separated seamounts (and the continental slope) may experience varying degrees of genetic isolation, resulting in high levels of endemism. While this hypothesis has been tested for multiple taxonomic groups in the Pacific, it has rarely been addressed in the Atlantic. We tested the null hypothesis that the geographic ranges of corals from NW Atlantic seamounts are restricted to individual seamounts. We examined 188 octocoral and 50 antipatharian colonies (representing 6 and 2 genera, respectively) from 14 seamounts, spanning 1700 km, and the adjacent continental margin and estimated their genetic variation using mitochondrial loci (*msh1* for all octocorals, as well as an intergenic region for isidids, and 3 multi-gene spanning segments for antipatharians). Well-sampled haplotypes were not geographically isolated on individual seamounts, thus refuting the hypothesis of local endemism of coral fauna on the New England and Corner Seamounts. The narrow geographic distribution of rare haplotypes is most likely due to undersampling rather than endemism. Our results do not preclude that cryptic variation and endemism not revealed by mitochondrial DNA may become evident should more variable markers be developed.

KEY WORDS: Endemism · Biogeography · Marine connectivity · Dispersal · Chrysogorgiidae · *Paramuricea* · Black coral · Anthozoa

INTRODUCTION

Seamounts, undersea mountains that rise from the sea floor, constitute a distinct deep-sea environment characterized by accelerated currents, exposed hard-substrates, and relatively high biomass and biodiversity (reviewed by Rogers 1994). It has been hypothesized that the topographic and hydrographic conditions associated with seamounts contribute to faunal isolation and the accumulation of highly endemic taxa (coined the 'seamount endemicity hypothesis' by McClain et al. 2009; see also Hubbs 1959, Wilson & Kaufmann 1987, Richer de Forges et al. 2000). While some studies have reported remarkably high levels of seamount endemism (e.g. Parin et al. 1997, Richer de Forges et al. 2000), recent reviews on seamount biodiversity warn that generalizations are difficult to make, since levels of endemism are wide-ranging and depend on the taxa and biogeographic regions under study (McClain 2007, Stocks & Hart 2007). Indeed, gaps in our understanding of faunal endemism on seamounts are mostly tied to undersampling across the diversity of seamount environments (e.g. depth range, steepness, geological origin, habitat composition and water column productivity) and geographical locations (e.g. latitude and isolation from continental margins). Of the 100 000 seamounts thought to exist worldwide (Kitchingman et al. 2007), only a few hundred have been biologically sampled (Stocks 2009), and most of these were only visited once (K. Stocks pers. comm.).

*Jana N. Thoma and Eric Pante have contributed equally to this project
**Corresponding author. Email: france@louisiana.edu

Therefore, gathering data allowing us to identify species pools, at local, regional and global scales, to determine the geographic distribution of seamount taxa and to establish faunal affinities between biogeographic regions is still central to seamount research.

McClain (2007) and Stocks & Hart (2007) suggested that a more synthetic understanding of the uniqueness of seamount fauna will come from (1) reducing sampling bias by visiting seamounts from a wider geographical range, (2) estimating endemism at different, well-defined spatial scales, and (3) incorporating molecular methods in the evaluation of faunal isolation. To date, research efforts have been highly skewed toward the Indo-Pacific region, and most of the data on Atlantic seamounts were gathered on northeastern chains (see Wilson & Kaufmann 1987, Mironov & Gebruk 2006, Stocks & Hart 2007 for a review of worldwide seamount endemism to mid-2005). The NW Atlantic, on the other hand, has been given very little attention. Also, most studies compare faunal assemblages from seamounts and slopes, or from seamounts belonging to different seamount chains. On the other hand, few studies focus on finer spatial scales, such as the geographical distribution of individual taxa within and among seamounts (Wilson & Kaufmann 1987, McClain 2007, Stocks & Hart 2007). Finally, most estimates of endemism on seamounts rely on the identification of specimens from morphological characters, which can be biased by phenotypic plasticity as well as convergent evolution and cryptic variation (e.g. Avise 2004).

The New England Seamounts (NES) and Corner Seamounts (CS) offer a suitable framework for the evaluation of the seamount endemicity hypothesis, even though their fauna have only recently begun to be documented (Moore et al. 2003, 2004 and references therein). These seamount chains were formed by a mantle-plume hotspot at the Mid-Atlantic Ridge and subsequent spreading of the seafloor (Sleep 1990), and extend for approximately 1700 km between the North American continental margin and Mid-Atlantic Ridge (Fig. 1). The oldest seamounts (e.g. Bear Seamount) are found in association with the continental margin (Duncan 1984), which also contains numerous canyons where hard substrates are exposed. Hydrography around the NES is affected by the flow of the Gulf Stream between Kelvin and Rehoboth Seamounts (e.g. Richardson 1981), and predicted current patterns show that the NES and adjacent continental margin may be hydrographically connected to each other but isolated from the eastern CS chain (Qiu 1994), although Bower et al. (2009) showed at least some deep subsurface floats flowed from the CS westward to the NES.

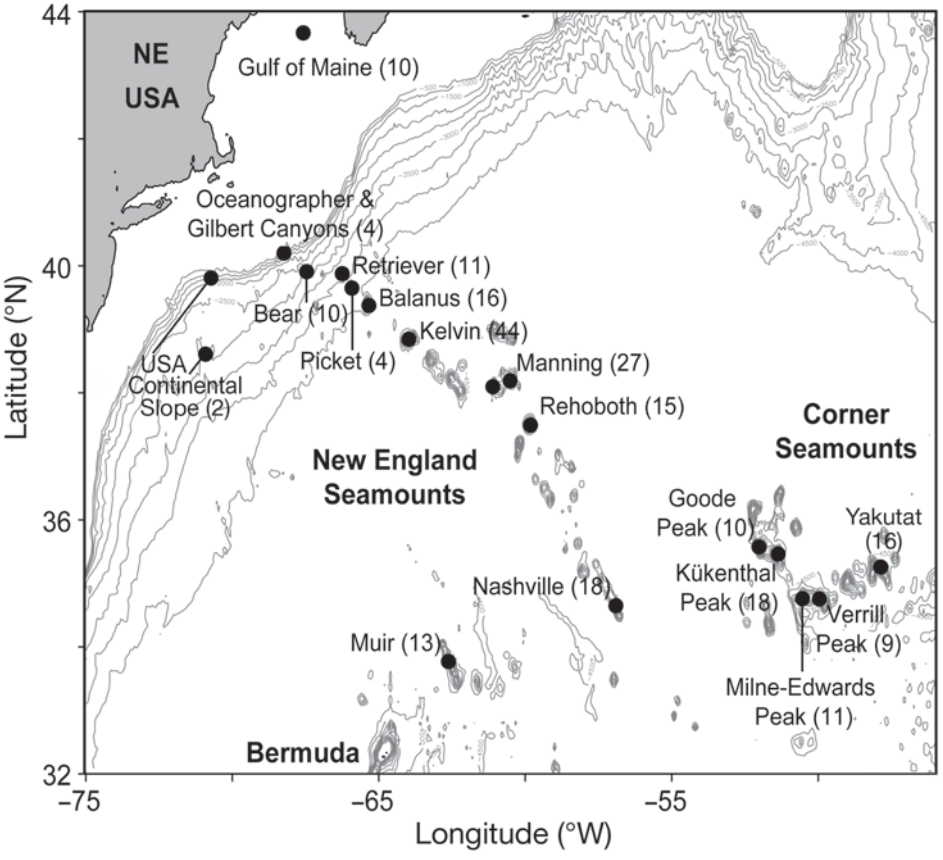

Fig. 1. Study region in the NW Atlantic. The total number of colonies collected and analyzed is given in parentheses for each collection site. Contour lines correspond to 500 m isobaths and extend to 4500 m

We characterized the geographic distribution of genetic types, or haplotypes, of representative octocorals and antipatharians within and across the NES and CS chains. Octocorals and black corals are common and conspicuous sessile fauna on seamounts (e.g. Rogers et al. 2007), which make them appropriate model systems for the evaluation of endemism on the NES and CS chains. Determining actual levels of endemism is impracticable, since it would require a complete catalog of deep-sea habitats and associated fauna. However, endemism of a particular taxon can be refuted if it is sampled outside of its hypothesized distributional area. Therefore, we evaluated the null hypothesis that haplotypes would be restricted, or endemic, to individual seamount peaks rather than being more widely distributed. We used mitochondrial (mt) DNA markers to avoid the confounding effects of phenotypic variation, cryptic variation and convergent evolution of morphological characters. In addition to geographically mapping the distribution of haplotypes, we constructed a haplotype network for *Paramuricea* (a particularly abundant and diverse octocoral genus in our study area) using specimens from within and outside the NW Atlantic to provide additional insight into biogeographic patterns. Our results provide information on the biodiversity, geographic distribution and inferred dispersal potential of octocorals and black corals, which may prove useful for future management and conservation of the NW Atlantic deep-sea benthic fauna.

MATERIALS AND METHODS

Sampling. Most specimens were collected during the Mountains in the Sea and Deep Atlantic Stepping Stones expeditions of 2003–2005 (NOAA Ocean Explorer expedition logs, http://oceanexplorer.noaa.gov/explorations/explorations.html). A total of 188 colonies of Octocorallia Haeckel, 1866 (3 families: Chrysogorgiidae Verrill, 1883: *Chrysogorgia* Duchassaing & Michelotti, 1864, *Iridogorgia* Verrill, 1883, *Metallogorgia* Versluys, 1902 and *Radicipes* Stearns, 1883; Isididae Lamouroux, 1812: *Acanella* Gray, 1870; and Plexauridae Gray, 1859: *Paramuricea* Kölliker, 1865) and 50 colonies of Antipatharia Milne Edwards & Haime, 1857 (1 family: Schizopathidae Brook, 1889: *Bathypathes* Brook, 1889 and *Parantipathes* Brook, 1889) were collected from 13 seamount peaks of the NES and CS chains, Muir Seamount and 5 locations on the adjacent continental margin of the eastern USA (200 to 2860 m depth) (Fig. 1, Table 1 in Supplement 1, see www.int-res.com/articles/suppl/m397p025_app.pdf). Additional samples from the North Atlantic, Gulf of Mexico and Pacific were used for wider biogeographic comparisons, including museum specimens collected from seamounts in the vicinity of the Azores that allowed for additional inter-seamount chain comparisons (Table S1).

To better distinguish incomplete sampling from true endemism, we plotted the geographic distribution of haplotypes—unique sequences of haploid mitochondria—as a function of sample size bounded by 2 theoretical expectations (Fig. 2): (1) local endemism (a haplotype is found on a single seamount peak independent of sample size) and (2) pan-distribution (the number of seamount peaks with a particular haplotype equals the number of seamount peaks sampled). We rejected the null hypothesis of seamount-scale endemism if haplotypes were found on more than one peak (see Fig. 2). Below the dotted line in Fig. 2 we cannot reject the null hypothesis that geographic restriction is due to endemism, as we cannot differenti-

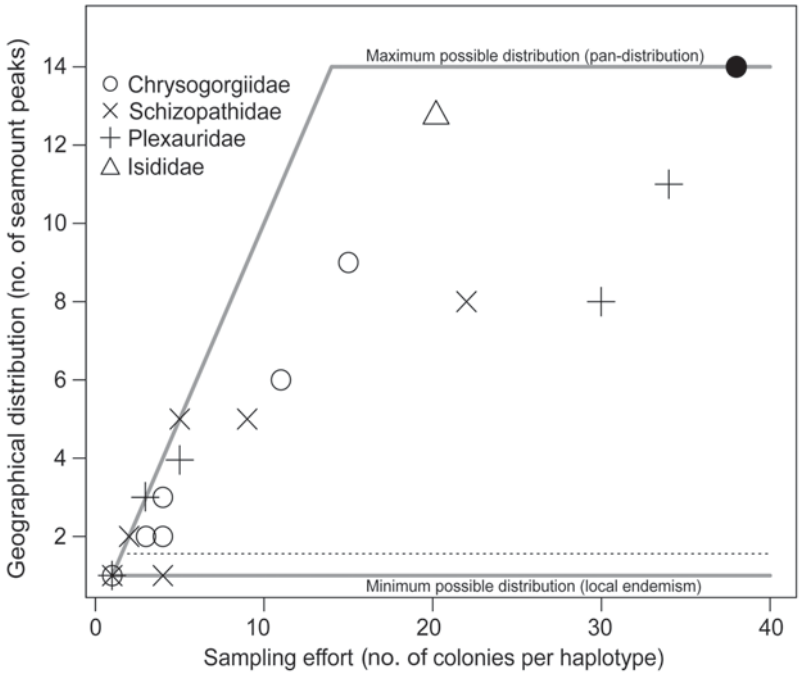

Fig. 2. Relationship between sampling effort and geographical distribution of haplotypes. Each point represents a particular haplotype; solid lines are theoretical boundaries (local endemism: haplotypes are restricted to a single seamount peak independent of sample size; pan-distribution: number of seamount peaks with a particular haplotype equals the number of colonies sampled, with an asymptote at the total seamount peaks sampled [present study, n = 14]). We reject the null hypothesis of endemism on individual seamounts if haplotypes are found on more than one peak (above dotted line). For example, the filled circle represents the single haplotype found for *Metallogorgia melanotrichos*, for which 38 colonies were sampled on 14 seamounts

ate endemism from undersampling. However, as sampling effort across seamounts increases and haplotypes are still found on one peak (i.e. the lower right part of the plot), the likelihood for endemism increases. It is worth noting that 3 of the seamounts sampled in the present study are characterized by multiple peaks that are separated horizontally by ~50 to 75 km by saddles that range from 1300 to 2300 m deeper than the summits (e.g. Milne-Edwards and Verrill Peaks comprise Caloosahatchee Seamount, Goode and Kükenthal comprise Corner Seamount). Although fauna on the peaks are topographically isolated, it can be argued that this isolation is less significant than for fauna on separate seamounts. However, we observed no case where haplotypes were restricted to only 2 peaks on the same seamount.

DNA extraction, PCR and sequencing. Total genomic DNA was extracted from both ethanol (EtOH)-preserved and frozen material using a CTAB (2% hexadecyltrimethylammonium bromide) protocol (with proteinase K in final concentration of 167 μg^{-1}) and a single chloroform-only extraction (modified from France et al. 1996).

To characterize endemism for a range of coral species on the NES and CS, we chose to sequence mtDNA as it presents few technical challenges and is cost-effective when studying species from a wide taxonomic range. Among mt markers that have been used in octocoral phylogenetics, a mismatch repair gene homolog (*msh1*) exhibits the highest rate of substitution and appears useful from the intrageneric to interordinal levels (16S, France et al. 1996; *msh1*, ND3 and ND4L, France & Hoover 2001; *cox1*, France & Hoover 2002, Calderón et al. 2006; ND2, ND3 and ND6, McFadden et al. 2004). Since *msh1* appears to lack intraspecific variation (e.g. Lepard 2003), we associate octocoral haplotypes with individual species. This same reasoning applies to the novel mt markers used for black corals (M. R. Brugler unpubl. data). Microsatellites, which are typically more variable than mtDNA and offer resolution to the population level, were not a viable option for the present study as they generally require species-specific optimization and a larger investment of time and money, as well as greater sample sizes.

Using PCR, we amplified the 5′-region of *msh1* for all octocorals plus, for the bamboo coral *Acanella*, a mt intergenic region and flanking sequence (*igr4*) (van der Ham et al. in press), and 5 partial genes, 1 tRNA and 3 mt intergenic regions (IGR) for black corals (Table 1). Each PCR reaction contained 1X TaKaRa *Ex Taq* buffer (Mg^{2+} free), 0.4 mM dNTPs, 1.5 mM of

Table 1. PCR primers used in the present study to amplify targeted gene regions, predicted fragment sizes (bp) using specified primer pairs and PCR cycle profiles

Amplified gene region Primer	Sequence 5′→3′	Fragment size (primer pairs)	Annealing temp./time	Extension temp./time	Source
msh1(5′) for Octocorallia					
(1) ND4L2475F	TAG TTT TAC TGG CCT CTA C	~990 (1&7)	51°C/20–30 s	72°C/50 s	Brugler & France (2008)
(2) ND42625F	TAC GTG GYA CAA TTG CTG	~845 (2&7)	51°C/30 s	72°C/60 s	Brugler & France (2008)
		~495 (2&6)	48°C/45 s	72°C/60 s	
(3) ND42599F	GCC ATT ATG GTT AAC TAT TAC	~870 (3&7)	45°C/45 s	65°C/60 s	France & Hoover (2002)
(4) CO3Bam5657F[a]	GCT GCT AGT TGG TAT TGG CAT	~1000 (4&7)	53°C/30 s	72°C/45 s	Brugler & France (2008)
(5) MSH3010F	GGA TAA AGG TTG GAC TAT TAT AG	~460 (5&7)	51°C/60 s	72°C/45 s	
(6) MSH3101R	GAT ATC ACA TAA GAT AAT TCC G				Sánchez et al. (2003)
(7) MUT3458R	TSG AGC AAA AGC CAC TCC				Sánchez et al. (2003)
cob(3′)-*igr4*-*nad6*(5′) for *Acanella*					
(8) CytbBam1279F	AGG AGC CAA TCC AGT AGA GGA ACC	~300 (7&8)	55°C/30 s	72°C/45 s	van der Ham et al. (in press)
(9) Nd6Bam1648R	TAY AGG TAA GAA ATG CGA GTG ATC				van der Ham et al. (in press)
cox3(3′)-IGR-*cox1*(5′) for Antipatharia					
(10) CO3gen3360F[b]	CTT TGT GGC AAC TGG GTT TCA TG	~1100 (9&11)	55°C/30 s	72°C/65 s	
(11) CO3anti3509F[c]	TGG TAT TGG CAT TTT GTG GAT GT	~950 (10&11)	53°C/30 s	72°C/60 s	
(12) CO1gen4446R	GAT AAC ATT GCA TAA ACC ATC CCT				
nad5-5′(3′)-IGR-*nad1*(5′) for Antipatharia					
(13) ND5-5′anti10725F	CAC ACT TGG TTG CCG GAT GCT ATG	~550 (12&13)	55°C/30 s	72°C/40 s	
(14) ND1anti11217R	CCT AAA ACC TTN CGT TCR GCT AAA GTT				
trnW-IGR-*nad2*(5′) for Antipatharia					
(15) TRPantiF	GGA AGA CCG TTA GCC TTC	~700 (14&15)	51°C/30 s	72°C/45 s	
(16) ND2anti1040R	CCA AAT AAG AAT AAG CCT GAA G				

[a]Primer used for *Acanella* only; [b]primer used for *Bathypathes* only, [c]primer used for *Parantipathes* only

$MgCl_2$, 0.24 µM of each primer (Operon Biotechnologies; Table 1), 2.5 µg of acetylated BSA (Promega), 0.5 U TaKaRa *Ex Taq* polymerase and 40 to 80 ng of genomic DNA, and was brought to a final volume of 25 µl with dH_2O. PCRs were run using the following cycle profile: initial denaturation at 94°C for 2 min followed by 30 to 40 cycles of denaturation at 94°C for 20 to 30 s, variable annealing and extension temperatures and times based on targeted gene region (Table 1) and a final extension at 72°C for 6 min. PCR products were purified by enzymatic digestion (2 U of *ExoI* and 0.2 U of shrimp alkaline phosphatase [Fermentas] per 1 µl of PCR product; Werle et al. 1994) or from low melting point (LMP) agarose by digestion with agarase (5 U per 100 µl melted 1% LMP agarose; Sigma-Aldrich).

Purified PCR reactions were cycle-sequenced using the ABI BigDye® Terminator v1.1 Cycle Sequencing Kit (1/4 reactions) and purified using either an EtOH/EDTA precipitation or Sephadex G-50 columns (Sigma-Aldrich). Purified products were electrophoresed on an ABI PRISM® 3100 or 3130*xl* Genetic Analyzer and sequence traces were edited using Sequencher™ v4.7 (Gene Codes). DNA sequences of specimens representing each haplotype, for each seamount peak, were submitted to GenBank (Table S1).

Genetic analysis. Representative haplotypes of *Paramuricea* were aligned using CLUSTAL-W (Thompson et al. 1994). This alignment was submitted to DnaSP v5.00.07 (Librado & Rozas 2009) to generate a haplotype sequence file; sites with missing data were excluded. A median-joining network was constructed using the DnaSP output file in Network 4.5.1.0 (Bandelt et al. 1999, www.fluxus-engineering.com).

RESULTS

Haplotype distribution and abundance on the NES and CS

Multiple haplotypes were found for 6 of the 8 genera analyzed; no haplotype diversity was observed among *Metallogorgia* and *Acanella* from our NW Atlantic col-

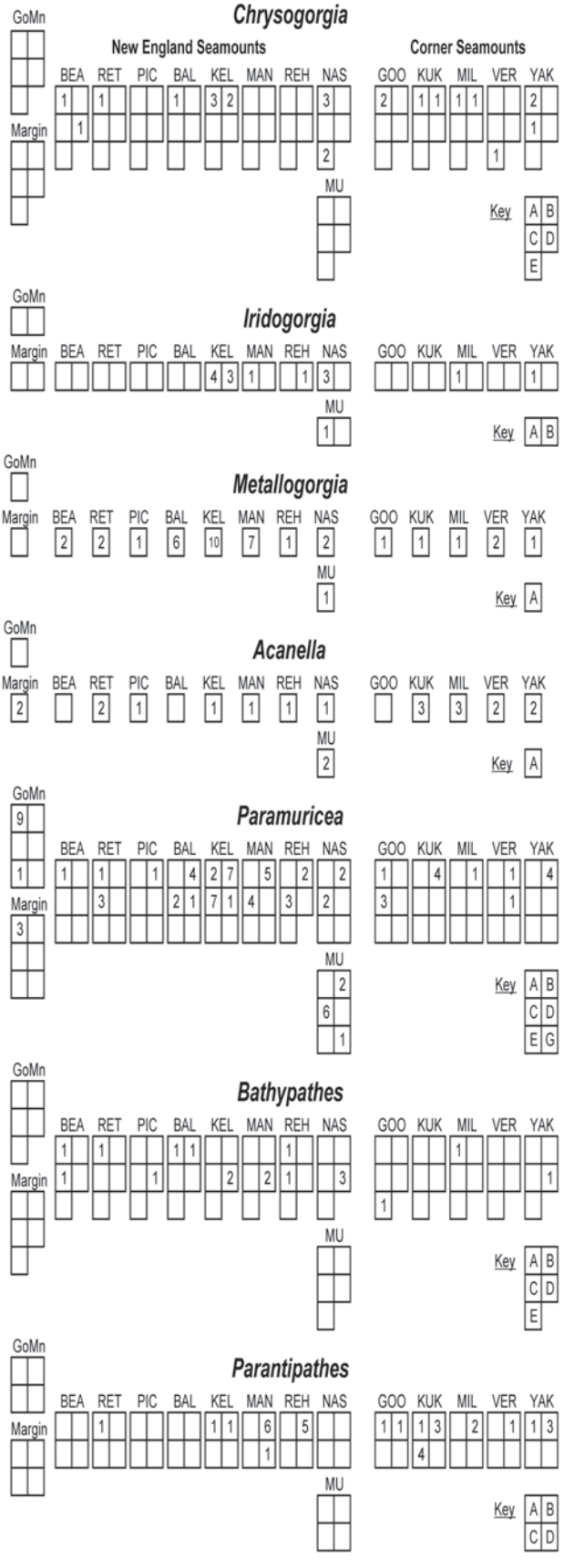

Fig. 3. Geographic distribution of haplotypes in the NW Atlantic. For each genus, sampling locations are shown by a group of 1 to 6 cells, each cell representing a haplotype (see keys). Numbers in cells are the number of colonies observed for a given haplotype. Locations are organized in a stylistic W–E and N–S orientation. GoMn: Gulf of Maine; Margin: Oceanographer and Gilbert Canyons, and 2 locations on the US Continental Slope; BEA: Bear Seamount; RET: Retriever Seamount; PIC: Picket Seamount; BAL: Balanus Seamount; KEL: Kelvin Seamount; MAN: Manning Seamount; REH: Rehoboth Seamount; NAS: Nashville Seamount; MU: Muir Seamount; GOO: Goode Peak; KUK: Kükenthal Peak; MIL: Milne-Edwards Peak; VER: Verrill Peak; YAK: Yakutat Seamount

Table 2. Number of colonies analyzed, N_C (from N_L locations); the gene region sequenced; sequence alignment length in bp, L (range of sequence lengths in bp); and haplotype diversity, N_H (no. colonies) from the NW Atlantic. Most variation in sequence length is due to missing data at the ends of the fragment analyzed, and not gaps in the alignment

Taxon	N_C (N_L)	Gene region	L	(Range)	N_H (No. of colonies)
Octocorallia: Chrysogorgiidae					
Chrysogorgia	24 (10)	*msh1*	697	–	A (15), B (4), C (1), D (1), E (3)
Iridogorgia	15 (7)		700	(697–700)	A (11), B (4)
Metallogorgia	38 (14)		697	–	A (38)
Radicipes	5 (3)		697	–	A (1), B (3), C (1)
Octocorallia: Isididae					
Acanella	21 (13)	*msh1*	705	(688–705)	A (21)
		igr4	290	–	
Octocorallia: Plexauridae					
Paramuricea	85 (16)	*msh1*	760	(642–760)[a]	A (17), B (33), C (30), D (3), E (1), G (1)
Antipatharia: Schizopathidae					
Bathypathes	18 (11)	*cox3*-IGR-*cox1*	833	(725–833)	A (5), B (1), C (2), D (9), E (1)
		IGR-*nad1*	405	(403–405)	
		IGR-*nad2*	528	(151–528)	
Parantipathes	32 (9)	IGR-*cox1*	820	(745–820)	A (5), B (22), C (4), D (1)
		nad5-5′-IGR-*nad1*	474	(415–474)	
		trnW-IGR-*nad2*	500	(207–599)	

[a]We could recover only partial *msh1* sequences (~208 bp) for the 3 specimens of *Paramuricea* from Oceanographer Canyon; for the region of overlap their sequences were identical to group A/D/E

lections (Fig. 3, Table 2). Nine out of 27 haplotypes were sampled only once (*Chrysogorgia* Types C and D, *Radicipes* Types A and C, *Paramuricea* Types E and G, *Bathypathes* Types B and E, and *Parantipathes* Type D). An additional 7 haplotypes were sampled from 4 or fewer colonies, but in only one case was a haplotype restricted to a single seamount peak (*Parantipathes* Type C on Kükenthal, n = 4). These 4 *Parantipathes* Type C colonies were collected within meters of one another during a single ROV dive. Four additional *Parantipathes* colonies were collected from different depths on the same peak, and corresponded to haplotype A or B, both of which were distributed across multiple seamounts (Fig. 3). Of the remaining undersampled haplotypes, 2 were found on 2 different seamounts within the NES (*Iridogorgia* Type B and *Bathypathes* Type C), 2 were found on 3 different seamounts in the NES and CS (*Chrysogorgia* Type B and *Paramuricea* Type D) and 2 were found on 2 different seamounts in the NES and CS (*Chrysogorgia* Type E and *Radicipes* Type B). The geographic range of a haplotype was positively correlated with the number times it was sampled, and for those haplotypes restricted to an individual seamount, sample size was low and we could not differentiate between true endemism and undersampling (Fig. 2). Though analysis of the vertical distribution of haplotypes was constrained to the varying depth range sampled for each seamount (Fig. S1), most haplotypes found on both the NES and CS chains, with the exception of *Parantipathes* Type B, do not appear to be stratified by depth.

Regional and global haplotype distributions

Of the 8 genera in the present study, 4 were collected on both the geographically isolated Muir Seamount and the NES and CS chains (Fig. 3). In only a single case did we observe a haplotype restricted to Muir Seamount: *Paramuricea* Type G, which was represented by one specimen. Type G is closely related to the widespread Type C, the most common haplotype found on Muir Seamount (Fig. 4).

We were able to sequence the 5′-region of *msh1* for 5 museum specimens collected in 1971 and 1993 from NE Atlantic locations. Three of the specimens had haplotypes that we identified on the NES and CS chains: *Iridogorgia* Type A was found on the SE side of San Jorge (Portugal), *Metallogorgia* Type A (n = 2) on Plato Bank, and *Chrysogorgia* type B on Irving Bank. A novel *Chrysogorgia* haplotype was collected from Tyro Bank (Type F). Although this haplotype was not found on the NES and CS chains, we recently collected a specimen with the same haplotype on the bathyal slope of the Bahamas (E. Pante & S. C. France unpubl. data). The 3 chrysogorgiid haplotypes that were common to the NES and CS chains and NE Atlantic were also found in the Pacific (Table S1).

Correspondence between genetic haplotypes and nominal species

Six of the 27 haplotypes correspond to nominal species. *Iridogorgia* Type A corresponds to *I. magnispiralis* Watling, 2007; Type B = *I. splendens* Watling, 2007;

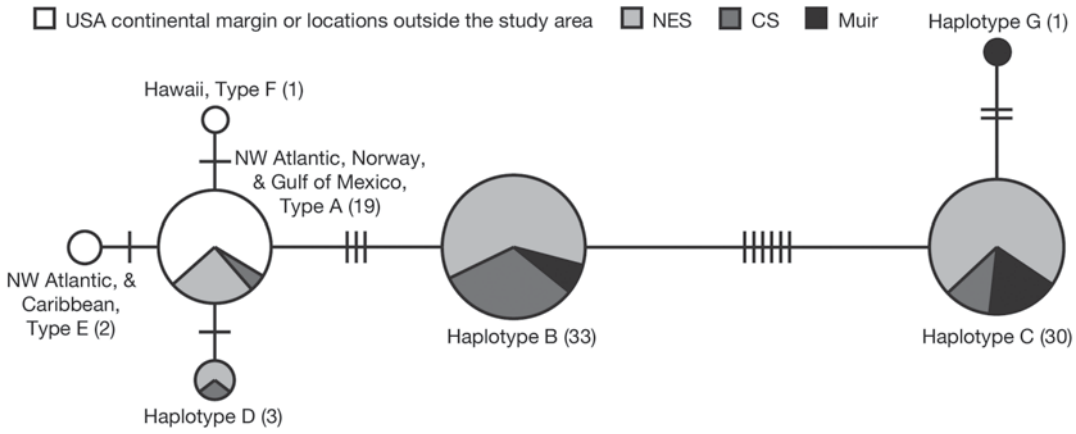

Fig. 4. Median-joining network depicting relatedness and geographic distribution of *msh1* haplotypes of *Paramuricea*, based on 14 variable sites. Circle size is proportional to the number of colonies with the corresponding haplotype (sample size indicated in parentheses). Hash marks and branch lengths represent the number of mutational steps between each haplotype

Metallogorgia Type A = *M. melanotrichos* (Wright & Studer, 1889); *Radicipes* Type A = *R. gracilis* (Verrill, 1884); *Acanella* Type A = *Acanella eburnea* (Pourtalès, 1868); *Bathypathes* Type D = *B. alternata* Brook, 1889. The remaining haplotypes come from colonies that are yet to be identified or are undescribed species: all types of *Chrysogorgia*; *Radicipes* Types B and C; all types of *Paramuricea*; *Bathypathes* Types A–C and E; and *Parantipathes* Types A–D.

Relationships among haplotypes of *Paramuricea*

We observed 6 haplotypes among the 85 colonies of *Paramuricea* sequenced from the NW Atlantic. Although there is little overall genetic differentiation among the haplotypes, they can be divided into 3 groups (Fig. 4). Types A and E, which are the only haplotypes found on the continental margin, group together with Type D (from Balanus, Kelvin and Verrill Peak). The remaining 3 haplotypes, which were collected from seamounts only, form 2 well-separated groups (Type B versus Types C/G). Levels of divergence among haplotypes are low within the A/D/E (uncorrected *p*-distances: 0.129 to 0.268%) and C/G (0.324%) groups. Types B and C/G are more divergent from A/D/E (0.528 to 0.667% and 1.544 to 1.861%, respectively) and from one another (1.055 to 1.238%) (Fig. 4).

We obtained *msh1* sequences from congeners collected from outside the study area (i.e. Norway, Gulf of Mexico, Caribbean and Hawaii). Specimens from Norway and the Gulf of Mexico were poorly preserved, and we could recover only partial *msh1* sequences (~208 bp); for the region of overlap their sequences were identical to group A/D/E. The sequence of the Caribbean specimen *Paramuricea multispina* (GenBank accession no. AY683077) is identical, except for a single ambiguity, to Type E found in a specimen from the Gulf of Maine. The ambiguous base is at a position in the alignment that is not variable in other sequences of *Paramuricea* or holaxonians examined (i.e. *Leptogorgia chilensis*, AY268460; *Pseudopterogorgia americana*, AY683087; *Eunicea clavigera*, AY683058). A complete *msh1* sequence from an unidentified *Paramuricea* collected in Hawaii (EU293799) yielded a novel haplotype (Type F). Both haplotypes E and F differ by a single substitution from Type A.

DISCUSSION

Improving geographic and taxonomic sampling

Based on samples from 13 seamount peaks from 2 chains spanning a distance of approximately 1700 km, the isolated Muir Seamount, and the adjacent continental margin, our study provides new data on the genetic diversity and geographic distribution of octocorals and black corals from a relatively understudied biogeographic region. To date, studies of faunal endemism on seamounts have largely been conducted in the South Pacific (Wilson & Kaufmann 1987, Stocks & Hart 2007). In Wilson & Kaufmann's (1987) comprehensive review of seamount biota, only 17% of the taxa included were from the Atlantic, among which only one specimen was collected in the vicinity of our study area (San Pablo Seamount, NES; Cairns 1982 in Wilson & Kaufmann 1987). Since 1987, several studies have characterized seamount biodiversity in the North Atlantic (Mironov & Gebruk 2006, review to mid-2005 by Stocks & Hart 2007), including Hall-Spencer et al. (2007), who estimated levels of endemism for corals in the NE Atlantic. Nonetheless, studies on seamount endemism in the NW Atlantic remain scarce (Sterrer

1998, Calder 2000, see also Mironov & Krylova 2006). This contribution, therefore, broadens the geographical breadth of the current body of research on seamount endemism.

The present study also provides data on the diversity of deep-sea corals, a relatively abundant and conspicuous, yet understudied, taxonomic group. Most studies of endemism have been conducted on fish, molluscs and crustaceans (Stocks & Hart 2007). Our study, along with Smith et al. (2004), represents one of the rare efforts to characterize the distribution of coral species across seamounts based on genetic tools (see Baco & Shank 2005 for a population-level study of Hawaiian deep-sea corals). While Smith et al. (2004) focused on a single octocoral subfamily (Keratoisidinae), the present study examines 3 families of octocorals as well as 2 antipatharian genera to provide information on distribution and diversity patterns from 2 distinct anthozoan lineages. We describe the distribution of 27 coral haplotypes, putatively corresponding to at least 27 species, only 6 of which could be identified based on published records. Two of these 6 identified species correspond to new species descriptions (Watling 2007) from specimens collected during the expeditions that underlie the present study. Taxonomic work on the remaining unidentified material is ongoing.

Biogeography of coral haplotypes

Seventeen of 27 coral haplotypes were not restricted to individual peaks, and 15 were not restricted to either seamount chain. For haplotypes that were restricted to individual peaks or chains, low sample size prevented us from discriminating true endemism from undersampling. In most studies that estimate endemism, the majority of taxa are not restricted to individual seamounts or seamount groups, which is evidence for successful dispersal and recruitment. In the NE Atlantic, <3% of octocorals, black corals and scleractinians are seamount specialists (Hall-Spencer et al. 2007); therefore, we expect levels of endemism on individual seamounts to be even lower. Similar results were observed in the Pacific, where no endemism was detected for bamboo corals (Smith et al. 2004). Since we frequently observed more haplotypes with increased HOV/ROV operation time (data not shown), increasing sampling effort could expand the observed geographical range of these rare haplotypes.

Three of the 27 haplotypes were also found in the Azores and in the Pacific. These wide geographic distributions could reflect high dispersal capabilities or ancient connections between ocean basins. The absence of variation among *Metallogorgia* colonies, for example, contrasts with the genetic diversity observed in other chrysogorgiid genera (i.e. *Chrysogorgia*, *Iridogorgia* and *Radicipes*). This pattern could reflect a relatively recent origin for the genus *Metallogorgia* (i.e. insufficient time to speciate), or superior dispersal capability relative to confamilial species. Deep-sea corals are long-lived (e.g. Roark et al. 2006, 2009) and slow-growing (Grigg 1993), and the time needed to diversify may therefore be relatively long (e.g. diversification and longevity are negatively correlated in the rockfish *Sebastes*; Bonsall 2006). Thus speciation rates may be slower and the generation of endemism through *in situ* diversification would be expected to take longer. Finally, the slow rate of evolution of the mitochondrial markers may not reflect actual species divergence. Fukami & Knowlton (2005) estimated the overall rate of mt genome evolution within the *Montastrea annularis* species complex (Scleractinia) at only 0.03 to 0.04% per million yr, and Lepard (2003) estimated the mutation rate of *msh1* to range between 0.14 and 0.25% per million yr, which renders it unlikely that substitutions will be seen between lineages that have diverged less than 1 million yr ago when comparing sequences of 1000 nucleotides or less.

Our collections of *Paramuricea* yielded a greater number of haplotypes (n = 6) than any other genus in our study area, and these could be divided into 3 divergent groups. One of these groups (A/D/E) was more closely related to colonies from distant locations (i.e. Norway, Gulf of Mexico, Caribbean and Hawaii) than to the other seamount haplotypes. The geographic paraphyly of NES and CS *Paramuricea* haplotypes, in combination with the observation of multiple, identical chrysogorgiid haplotypes found in both the Pacific and Atlantic, suggests that the 2 seamount chains are not discrete biogeographic units. A similar trend is observed for taxa of the Reykjanes Ridge (North Atlantic), which also occur in the Pacific and Antarctic (Mironov & Gebruk 2006).

Study limitations and future efforts

Reviewers of seamount endemism (McClain 2007, Stocks & Hart 2007) have identified geographically uneven sampling efforts and heavy reliance on morphology-based systematics as factors potentially preventing us from accurately estimating endemism at multiple spatial scales and reaching a synthetic understanding of the mechanisms involved in faunal isolation. For example, we observed that the divergent *Paramuricea* haplotypes come from colonies that are morphologically indistinguishable when viewed *in situ*. In the present study we provide molecular-based distributional information on an understudied taxonomic group from an underexplored area. Our results do not

support seamount-scale endemism in the NW Atlantic, as well-sampled haplotypes were not geographically restricted to individual seamounts and, in some instances, haplotypes also occurred in the Pacific. Our efforts, however, are limited by gaps in the taxonomy of the concerned groups, the scarcity of samples outside of the NES and CS chains, and the potential lack of fine systematic resolution offered by currently available molecular markers.

Future efforts should focus on determining the correspondence between haplotypes and nominal species. As previously stated, preliminary efforts suggest that *msh1* is appropriate for detecting variation at the species level, but ultimately, it will be necessary to formally describe all specimens collected on the NES and CS chains to establish congruence between molecular and morphological data.

At the evolutionary time-scale considered, more intensive sampling will be necessary to establish biogeographic affinities between seamount chains. Using only a few specimens from museum collections, we were able to establish that some haplotypes occur outside of the NW Atlantic. Only with sampling efforts spanning ocean basins will we be able to establish how common widely distributed taxa are. For example, samples collected during a recent expedition to the bathyal slope of the Bahamas (Bahamas Deep Corals 2009, http://oceanexplorer.noaa.gov/explorations/09deepseacorals/) broaden the geographical distribution of 6 haplotypes belonging to the Chrysogorgiidae (E. Pante & S. C. France unpubl. data), 2 of which had particularly narrow ranges on the NES and CS (i.e. *Iridogorgia* Type B, *Chrysogorgia* Type C).

Developing molecular markers that are informative at the population level will provide a more complete understanding of the mechanisms involved in isolating taxa from different seamounts and seamount chains. Short sequences (<1000 bp) of mtDNA have not been shown to be variable at the intraspecific level in most anthozoans and therefore are not suitable for population-level studies (France & Hoover 2001, 2002, Shearer et al. 2002, McFadden et al. 2004, but see Chen et al. 2008a,b for scleractinians). Nuclear DNA, however, may be more variable than mtDNA in anthozoans (Chen et al. 2009). Low-copy nuclear markers have been used for species delineation and detection of introgression in stony corals (Hatta et al. 1999, van Oppen et al. 2000, 2001, 2004, Vollmer & Palumbi 2002, 2007) and soft corals (Concepcion et al. 2008), and could be particularly informative for those species that might have diverged less than 1 million yr ago. Microsatellites have been used to study population-level questions in deep-sea corals (e.g. Baco & Shank 2005), but they require high sample sizes. Sampling constraints in deep-sea hard-substrate communities render abundant collections of targeted taxa a difficult goal to achieve, particularly on expeditions with multiple objectives; we rarely were able to collect more than 5 colonies of a species per seamount peak.

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