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Characterizing population structure of coral-associated fauna from mesophotic and shallow habitats in the Caribbean

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Symbiotic relationships are a common phenomenon among marine invertebrates, forming both obligatory and facultative dependencies with their host. Here, we investigate and compare the population structure of two crustacean species associated with both shallow and mesophotic ecosystems: an obligate symbiont barnacle (Ceratoconcha domingensis)*, of the coral* Agaricia lamarcki *and a meiobenthic, free-living harpacticoid copepod* (Laophontella armata)*. Molecular analyses of the Cytochrome Oxidase Subunit I* (*COI*) *gene revealed no population structure between mesophotic and shallow barnacle populations within south-west Puerto Rico* ($\Phi_{ST} = 0.0079$, $P = 0.33$)*. The absence of population structure was expected due to the pelagic naupliar larvae of the barnacles and the connectivity patterns exhibited by the coral itself within the same region.* Laophontella armata *exhibited significant structure based on the mitochondrial COI gene between the mesophotic reef ecosystem of El Seco, Puerto Rico and mangrove sediments of Curaçao* ($\Phi_{ST} = 0.2804$, $P = 0.0$)*. The El Seco and Curaçao copepods shared three COI haplotypes despite the obligatory benthic development of harpacticoid copepods and the geographic distance between the two locations. Three other COI haplotypes from El Seco exhibited higher than expected (up to 7%) intra-species variability, potentially representing three new cryptic species of harpacticoid copepods or rare, deeply divergent lineages of* L. armata*. This result is evidence for the urgent need of a deeper investigation into the meiofauna diversity associated with mesophotic coral ecosystems* (*MCEs*)*, arguably the most diverse metazoan component of MCEs.*

Keywords: Mesophotic coral ecosystems, biodiversity, Caribbean, harpacticoid copepods, COI, population connectivity

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INTRODUCTION

Scleractinian corals represent the outcome of intricate symbioses between the coral host and a diverse group of *Symbiodinium* spp. (zooxanthellae), endolithic algae, fungi, bacteria, archaea, protists, viruses and associated macro/meiofauna (Rohwer *et al.*, 2002; Kramarsky-Winter *et al.*, 2006; Sekar *et al.*, 2006), collectively known as the holobiont. The baroque three-dimensional characteristics of coral reefs create a hotspot for these symbioses while housing extraordinary biodiversity, largely dominated by smaller invertebrates (Buhl-Mortensen *et al.*, 2010; Knowlton *et al.*, 2010). These invertebrates often live in complex interdependencies with scleractinian corals, relying on the corals for food, habitat and settlement space (Glynn & Enochs, 2011; Stella *et al.*, 2011). By utilizing corals for such close partnerships, many reef invertebrates have become reliant on coral substrate, a large portion even exhibit high degrees of specialization and develop lifelong associations (Blackall *et al.*, 2015); these interdependencies are the result of millions of years of co-evolution. Symbiotic relationships can either be obligate (must live on their host to survive) or facultative (may live on their host, but do not have to for survival) (Connor, 1995; Stella *et al.*, 2011). The forms of symbiosis and species relationships will have profound consequences for the overall functioning, ecology and dynamics of the coral reef (Nelson *et al.*, 2016), which could create forces (e.g. micro-niche specialization) that drive genetic divergence and over time lead to sympatric speciation by host shift (Tsang *et al.*, 2009).

One of the most visible and ubiquitous coral associates are the barnacles, which as adults can be found partially embedded in the coral tissue. Darwin's own (1859) seminal work on speciation patterns of coral barnacles first highlighted this important association in the marine environment. More recently, Mokady *et al.* (1999) examined the genetic diversity of two genera of coral-inhabiting barnacles. The *Cantellius* genus had low genetic variability and moderate phenotypic plasticity over a large host range, inhabiting corals from three families. The *Savignium* genus displayed large morphological and genetic variation within and between species,

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clustering phylogenetically according to coral host, indicating strong co-speciation patterns between the coral host and the barnacles. The genetic differences between the two barnacle genera correlated with differences in life-history parameters such as host-infestation, reproduction and larval trophic type.

The Copepoda have developed more associations with marine animals than any other group of Crustacea (Humes, 1985; Stock, 1987, 1988; Ivanenko, 1998), accounting for ~363 coral associated species in 99 genera, 19 families and three orders (Cheng *et al.*, 2016). Coral-associated copepod abundance and presence seem to be determined by specific life history and reproductive patterns, surface area of host coral colony and nitrate and chlorophyll-a concentrations in the ambient seawater. There are no population studies of coral associated copepods since there is still a vast number of undescribed coral-inhabiting species. However, population structure has been revealed on small and large geographic scales for harpacticoid copepods living in high intertidal rock pools and mudflats (Burton *et al.*, 1979; Schizas *et al.*, 2002; Denis *et al.*, 2009; Willett & Ladner, 2009; Gregg *et al.*, 2010).

Population structure of coral-associated fauna remains an important yet understudied subject for reef ecologists. The need to discern gene flow and speciation patterns between shallow and mesophotic reefs (MCEs – photosynthesis-based reefs developing between 30 and 100 m) has become a paramount objective for marine conservationists. Shallow-water corals are experiencing dramatic population declines from temperature-induced bleaching and disease outbreaks linked to global warming, compounded with local anthropogenic stressors (Gardner, 2003; Carpenter *et al.*, 2008; Harvell *et al.*, 2009). Congruently, as coral reefs diminish so too does available substrate for coral reef-associated invertebrates and the persistence of metapopulations may depend on periphery subpopulations that extend to the edge of a species' range (Holstein *et al.*, 2015). MCEs may better withstand disturbances experienced on shallow-water reefs due to their increased distance from land and relatively more stable temperatures (Hinderstein *et al.*, 2010; Slattery *et al.*, 2011; Kahng *et al.*, 2014, 2016). MCEs could be vital refuge habitats for coral reef-associated invertebrates and could act as stepping stones between shallow and deeper environments. Therefore, understanding gene flow between coral reefs from a wide range of species with different degrees of association is important for conservation and management of resident coral reef biodiversity.

The motivation to include non-coral MCE-associated fauna stems from the complete lack of data on this significant component of mesophotic habitats and its potential to provide a different perspective from previous studies focusing on corals themselves. Thus, this study aims to better understand connectivity of reefs with representatives from two crustacean taxa displaying different association types to corals. We estimated population level characteristics of (i) the barnacle *Ceratoconcha domingensis* (Des Moulins, 1866), an obligate symbiont of the coral *Agaricia lamarcki* (Milne Edwards & Haime, 1851), from shallow and mesophotic reefs in La Parguera and Guánica, Puerto Rico and (ii) the free-living harpacticoid copepod, *Laophontella armata* (Willey, 1935), from a mesophotic coral ecosystem in El Seco (Vieques, Puerto Rico) and a mangrove habitat in Curaçao (Figure 1; Table 1). Barnacles were extracted from *A. lamarcki* since the coral has a wide geographic range in the Caribbean basin and a recorded depth distribution from 10 to 75 m (i.e. depth-generalist, Aronson *et al.*, 2008). This plate-shaped coral also has pronounced levels of population connectivity across the same geographic range where the barnacles were collected from Hammerman *et al.* (2017).

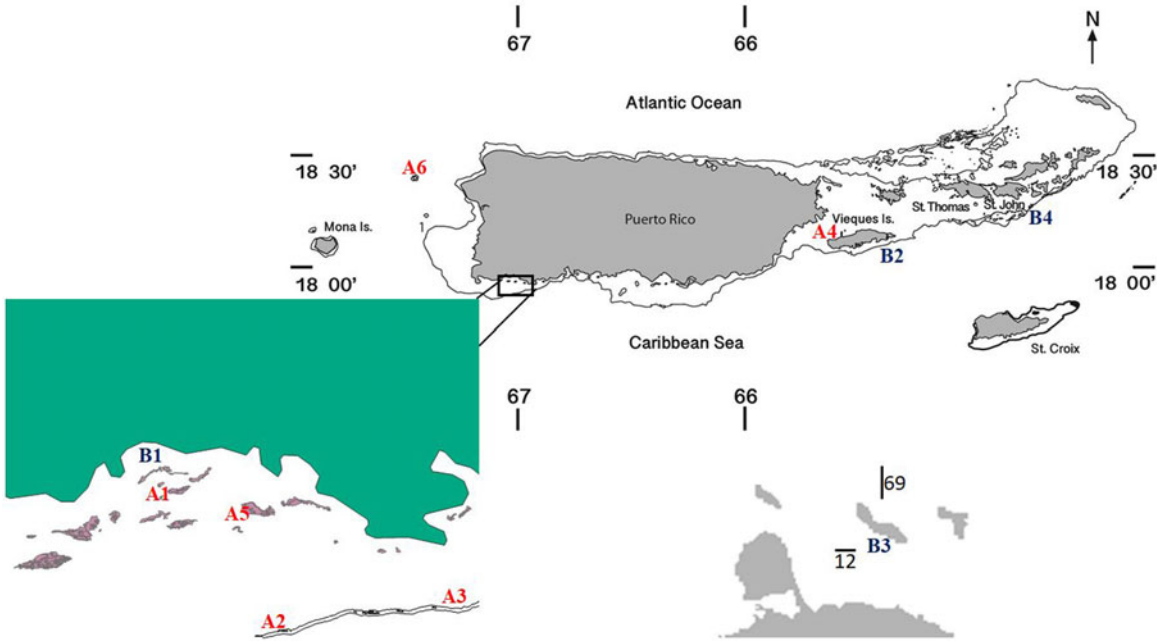

Fig. 1. Site map for barnacle *Ceratoconcha domingensis* and copepod *Laophontella armata* collections across the US Caribbean and Curaçao: Barnacle sites (red), A1 (Inner La Parguera, Puerto Rico), A2 (La Parguera Shelf Edge, Puerto Rico), A3 (Guánica Shelf Edge, Puerto Rico), A4 (Vieques, Puerto Rico), A5 (Outer La Parguera, Puerto Rico) and A6 (Desecheo, Puerto Rico); Copepod sites (blue), B1 (La Parguera Shallow, Puerto Rico), B2 (El Seco Mesophotic, Puerto Rico), B3 (Curaçao Shallow) and B4 (St. John Mesophotic, US Virgin Islands).

Table 1. Collection information and diversity indices for the barnacle (*Ceratoconcha domingensis*) and copepod (*Laophontella armata*) sample sets.

Sample locality	Latitude	Longitude	Depth (m)	*n*	Hap (no. of haplotypes)	Haplotype diversity, *h*	Theta	Pi	Tajima's *D*	Fu's *Fs*
Ceratoconcha domingensis (Obligate barnacle)										
Inner La Parguera	17.95	−67.05	18–22	33	23	0.966 ± 0.0034	0.0122	3.87 ± 2.22	−1.62 ($P = 0.031$)	−12.894 ($P = 0.00$)
La Parguera Shelf Edge	17.89	−66.97	24–42	17	13	0.949 ± 0.044	0.014	5.17 ± 2.95	−1.61 ($P = 0.044$)	−4.945 ($P = 0.013$)
Guánica Shelf Edge	17.9	−66.92	24–38	18	12	0.0017 ± 0.041	0.0112	4.85 ± 2.77	−1.083 ($P = 0.144$)	−3.447 ($P = 0.048$)
Vieques	18.12	−65.43	20	4	3	0.833 ± 0.222	N/A	2.83 ± 2.23	0.372 ($P = 0.735$)	0.646 ($P = 0.547$)
Outer La Parguera	17.93	−67.01	18–22	15	13	0.981 ± 0.031	0.0125	5.2 ± 3.0	−1.227 ($P = 0.1004$)	−6.39 ($P = 0.002$)
Desecheo	18.38	−67.47	70	6	6	1.00 ± 0.096	N/A	8.4 ± 5.24	−0.804 ($P = 0.271$)	−1.232 ($P = 0.140$)
Laophontella sp. (Loosely associated copepod)										
La Parguera Shallow	17.96	−67.04	0	2	1	0	N/A	0.00 ± 0.00	0 ($P = 1.0$)	N/A
El Seco Mesophotic	18.12	−65.17	52–67	33	18	0.926 ± 0.032	0.029	7.564 ± 4.028	−0.978 ($P = 0.152$)	−3.07 (0.131)
Curaçao Shallow	12.06	−68.85	1	17	4	0.618 ± 0.106	0.0045	1.971 ± 1.311	0.37 ($P = 0.679$)	1.479 ($P = 0.804$)
St. John Mesophotic	18.22	−64.67	53	2	2	1.00 ± 0.5	N/A	6.00 ± 6.481	0 ($P = 1.0$)	1.79 ($P = 0.52$)

MATERIALS AND METHODS

Sample collections and DNA extraction

The mesophotic (>30 m) samples of copepods from El Seco, Vieques were obtained through previous mesophotic research cruises conducted by the Department of Marine Sciences at the University of Puerto Rico at Mayagüez (UPRM-DMS). The copepod specimens were collected along with other meiofauna and macrofauna after washing mesophotic coral fragments and other substrata overtop a 0.125 mm mesh sieve. All organisms were placed in 50 ml vials and fixed in absolute ethanol. Copepod samples were handpicked with fine tweezers after being observed and identified under a dissection microscope. The species identification of the copepods was cross-checked against the literature after dissections of a few specimens. The shallow samples of *L. armata* were collected opportunistically in Curaçao from fine sediment below mangrove roots. These collections consisted of sieving loose sediments near mangrove roots with a 0.500 mm on top of a 0.125 mm mesh sieve. Again, the subsequent samples were fixed, examined under microscope and prepared for DNA extraction. DNA extraction for the copepods consisted of placing a whole specimen into a 1.5 ml DNA- and RNA-free microcentrifuge tube and using the Chelex protocol (Sigma Aldrich). The Chelex solution consisted of 0.25 g of Chelex per 3 ml of PCR grade water. The tissue samples were submerged in 20 µl of Chelex solution and incubated on ice for 15 min. Following incubation, the samples were vortexed for 30 s, and then incubated for 20 min at 95 °C. The tube was centrifuged for three minutes at 12,000 rpm and the supernatant was pipetted off without disturbing the pellet.

Barnacles were removed from mesophotic (>30 m) and shallow (<18 m) *Agaricia lamarcki* colonies in south-west Puerto Rico (La Parguera and Guánica), Vieques (an island east of Puerto Rico) and Desecheo (an island off the west coast of Puerto Rico) using scuba with a chisel and hammer (Figure 1). Sampling in La Parguera resulted in three groupings based on distance from land (Inner, Outer and Shelf Edge La Parguera) and one grouping for Guánica (Guánica Shelf Edge, Table 1). In the lab, barnacle samples were stored in 50 ml conical vials in 95% ethanol in a −20°C freezer until molecular processing. The barnacles were macerated in a mortar and by pestle to a fine pulp and DNA extraction followed standard protocols as outlined by the Qiagen DNeasy Plant and Tissue Mini Kit (Qiagen, Valencia, CA). All DNA templates were inspected for quality on 2% agarose gels and with the Nanodrop Spectrophotometer 2000.

PCR amplification and sequencing preparation

Copepod and barnacle partial Cytochrome C Oxidase Subunit I (COI) was PCR-amplified. Primers specifically designed for *Laophontella armata* were used to amplify the COI gene, after initial amplification with the universal COI primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). The new species-specific primers were designed with Primer3 and are as follows: LaophontellaF (5′-ACA ATG TGG TAG TAA CTG CTC-3′) and LaophontellaR (5′-GAT CCC CTC CAC CTC TTA CG-3′). Each individual PCR reaction contained 12.5 µl MyTaq^{TM} Mix (2×, Bioline), 0.3 µl LaophontellaF primer, 0.3 µl LaophontellaR primer, 4.5 µl distilled H_2O

and 7.0 µl DNA template. The PCR conditions for the copepod DNA were 95°C for 1 min, followed by 35 cycles each at 95°C for 30 s, 47°C for 30 s, and 72°C for 1 min, with a final extension step at 72 °C for 7 min. Barnacle COI was amplified using the universal primers LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′). Prior to DNA amplification, the extracted barnacle DNA was 1/10 diluted. Individual reactions then consisted of 0.3 µl LCO1490, 0.3 µl HCO2198, 8.0 µl of distilled H_2O and 4.0 µl of diluted barnacle DNA template. Cycle conditions for barnacle amplification were as follows: 94°C for 2 min, followed by 35 cycles at 95°C for 15 s, 45.5°C for 50 s and 72°C for 1 min, with a final extension at 72 °C for 5 min. All positively confirmed copepod and barnacle PCR amplicons were purified using 4.0 µl of ExoSAP-IT PCR cleanup reagent (Thermo Fisher Scientific) per 5 µl of PCR product. Samples were plated on 96-well sequencing plates and were processed for Sanger sequencing in both directions using the Big Dye 3.1 Terminator Cycle Sequencing Kit and the ethanol-precipitated products were loaded into an ABI 3130xl 16-capillary Genetic Analyzer at Yale University. All DNA sequences have been submitted to GenBank (*Ceratoconcha domingensis* COI: accession numbers MH347748–MH347839; *Laophontella armata* COI: accession numbers MH347840–MH347893).

The DNA chromatograms produced were visually inspected for quality and accuracy in nucleotide base assignment in Codon Code Aligner v. 5.1.5 (Codon Code Corp.). Sequences were trimmed in Codon Code Aligner then aligned by the MAFFT Algorithm v. 7 (Katoh & Standley, 2013) for further analyses. Substantiated sequences were imported into DNAsp v. 5.10.01 (Librado & Rozas, 2009) and clustered based on sample locality. An ARLEQUIN haplotype file was generated by DNAsp for downstream genetic diversity and population structure analyses. Statistical selections of best-fit models of nucleotide substitution for both datasets were performed using jModelTest 2.1.10 (Darriba *et al.*, 2011).

Genetic diversity and population structure

Molecular diversity indices for copepod and barnacle COI sequences, including the number of variable sites (*S*), number of haplotypes (Hap), haplotype diversity (*h*) and the mean number of nucleotide differences between haplotypes (*k*) were calculated for each sample locality in DNAsp. Nucleotide diversity (π), neutrality tests (Tajima's *D* and Fu's *Fs*), and population structure (AMOVA) were estimated using the software ARLEQUIN v. 3.5.2.2 (Excoffier & Lischer, 2010). All analyses done in ARLEQUIN were performed using the Tamura–Nei model of nucleotide substitution (Tamura & Nei, 1993) determined by jModelTest2 with 10,000 permutations. Pairwise Φ_{ST} population comparisons were done between all sample locations (Weir & Cockerham, 1984) as implemented in ARLEQUIN and statistical significance was tested against the null hypothesis of panmixia. Haplotype networks were illustrated with a median-joining network algorithm ($\varepsilon = 0$) (Bandelt *et al.*, 1999) using the software PopART v. 1.7 (Leigh & Bryant, 2015) to analyse haplotype genealogy. Sequence divergences between populations were estimated in PAUP (Swofford, 2001).

RESULTS

Ceratoconcha domingensis (Cirripedia: Balanomorpha: Pyrgomatidae)

COI sequences (680 bp) were obtained from 92 individual barnacles, representing six different sample locations (Table 1). The locality with the most samples was Inner La Parguera (N = 32, depth 18–22 m) and the lowest was Vieques (N = 4, depth 20 m). From these 92 sequences, we identified 48 COI haplotypes, 37 of which were singletons. Desecheo (depth 70 m) had the highest haplotype diversity as all sequences were different ($h = 1.00$); however, the sample size was small (N = 6). Inner and Outer La Parguera (both sites at depth 18–22 m) had larger sample sizes (N = 32 and N = 15, respectively) and still displayed high haplotype diversity, 0.966 and 0.981 respectively (Table 1). The lowest haplotype diversity was observed in the Guánica Shelf Edge ($h = 0.002$; depth 24–38 m; Table 1). The sample locality represented by the highest number of COI haplotypes was Inner La Parguera (N = 23), while the lowest was Vieques (N = 3). Haplotype 11 was the most abundant (N = 14) throughout our sample set, found at all locations except Desecheo (Figure 2). Haplotype 11 was most common in Inner La Parguera (N = 6) and the La Parguera Shelf Edge (N = 4, depth 24–42 m). Haplotype 7 (N = 6) was exclusively found in La Parguera, though most commonly in Inner La Parguera (N = 3).

The haplotype network for barnacle COI sequences does not indicate the presence of any distinct sequence clusters by geography, suggesting the absence of population structure among the sampled locations (Figure 2). Nucleotide diversity (π) values were highest for La Parguera Shelf Edge (0.0083) and Outer La Parguera (0.0082). Similarly, the highest values of θ were observed in the La Parguera Shelf Edge (0.0130) and Outer La Parguera (0.0108). The Desecheo and Vieques samples represented six and four of the 48 haplotypes, respectively, though due to the small sample size and high similarity with the other locations, they were not considered while discussing π and θ values per site (Table 1). The excess of singletons resulted in rejecting the equilibrium hypothesis for all sample locations (Fu's *Fs*) and Inner and La Parguera Shelf Edge (Tajima's D) was statistically significant (Table 1). AMOVA results indicated that there was no population structure ($\Phi_{ST} = 0.00797$) between the sample localities (Table 3). Pairwise Φ_{ST} comparisons confirmed the AMOVA results showing no significant differences among the sampled locations (Table 2).

Laophontella armata (Copepoda: Harpacticoida: Tetragonicipitidae)

Laophontella armata samples yielded 54 COI sequences (496 bp) from four different locations (Table 1). Sample locations La Parguera Shallow and St. John Mesophotic both were represented by two sequences each, and while included in the PoPArt analyses, they were not considered further. The considered data set consisted of 50 *Laophontella* COI sequences from El Seco Mesophotic (N = 33) and Curaçao Shallow (N = 17). Of the 50 sequences, 20 were identified as haplotypes and of those, 11 were singletons (El Seco Mesophotic, N = 18; Curaçao, N = 2, Figure 3). Haplotype diversity was

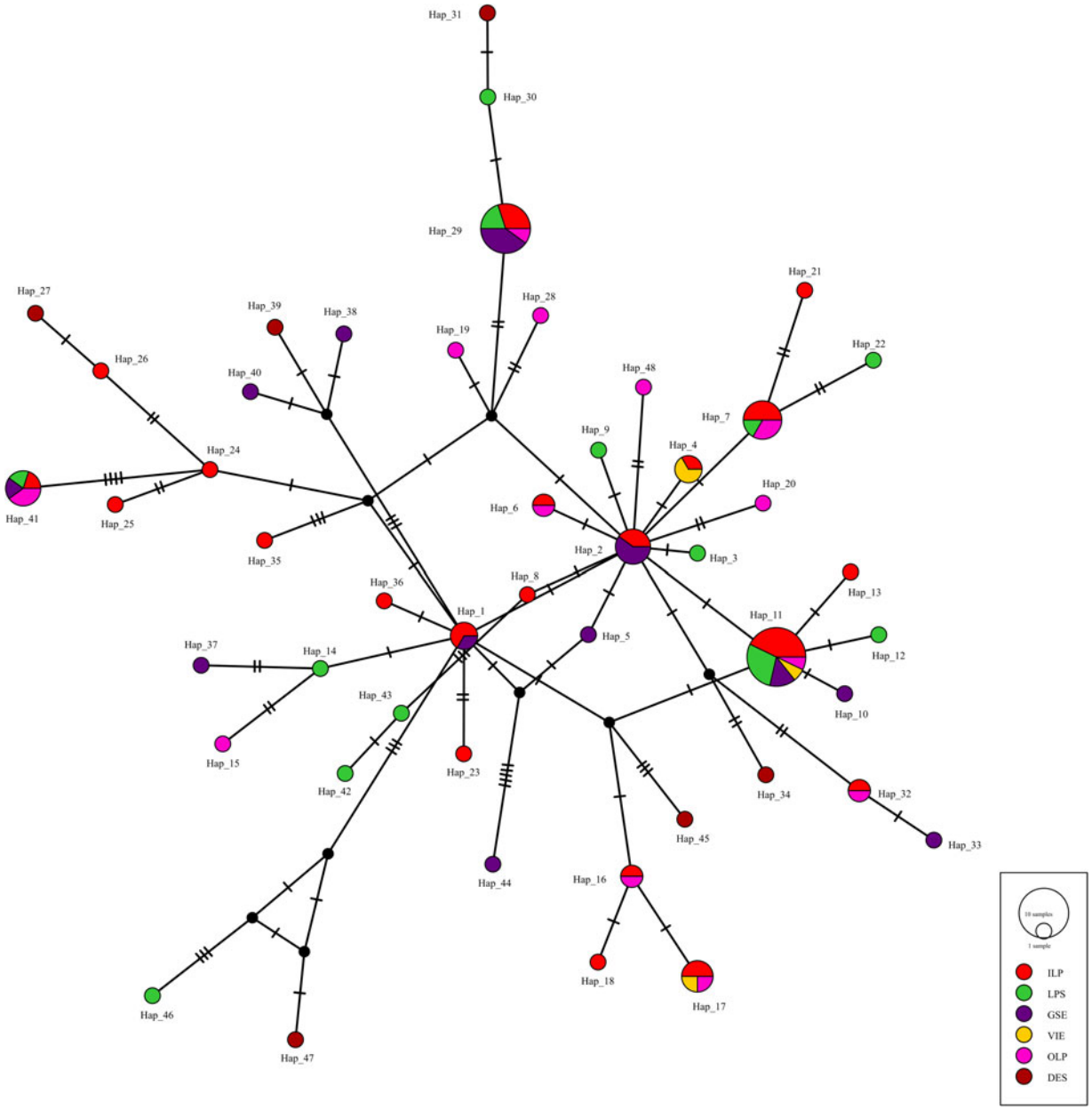

Fig. 2. Median-joining network based on *Ceratoconcha domingensis* COI haplotypes generated in this study. The network was estimated using the median-joining algorithm in PoPArt with epsilon = 0. Each circle represents a different haplotype and the size of a circle correlates with number of individuals belonging to that given haplotype. Colours indicate the geographic origin of sequences as follows: i. Inner La Parguera (ILP), red; ii. La Parguera Shelf Edge (LPS), green; iii. Guánica Shelf Edge (GSE), purple; iv. Vieques (VIE), yellow; v. Outer La Parguera (OLP), pink; vi. Desecheo (DES), maroon.

higher in El Seco Mesophotic ($h = 0.926$) than Curaçao Shallow ($h = 0.618$, Table 1). Haplotype 17 was the most common haplotype (N = 10) and was exclusively found in Curaçao Shallow. Haplotype 18 (N = 8) was the second most common haplotype and was unique to El Seco Mesophotic. COI haplotypes 2 and 6 were the only shared haplotypes between El Seco Mesophotic (N = 1 for both haplotypes) and Curaçao (N = 4 for Haplotype 2, and N = 6 for Haplotype 6).

The haplotype network did not indicate any distinct clustering by geography except the exclusive occurrence of Haplotypes 17 and 18, in Curaçao and El Seco Mesophotic, respectively (Figure 2). The highest π and θ values were observed in the El Seco Mesophotic sample set, 0.015 and 0.029, respectively. Neutrality tests Tajima's *D* and Fu's *Fs* were found to be insignificant indicating that the nucleotide patterns of variation are consistent with the neutral theory of evolution. Pairwise Φ_{ST} comparison between El Seco Mesophotic and Curaçao Shallow indicate a strong population structure between the two locations (Table 2). Surprisingly, within the El Seco Mesophotic sample set there were three divergent haplotypes (Haplotypes 21 (N = 1), 22 (N = 1) and 23 (N = 2)) that could represent three cryptic species of *Laophontella*. The divergent sequences are not obvious numts since they do not contain stop codons and match other harpacticoid copepod sequences already present in GenBank, therefore we suggest that they represent harpacticoid copepod sequences. Pairwise Φ_{ST} comparisons were performed including and excluding these sequences, in both cases Φ_{ST} values showed structure between El Seco and Curaçao

Table 2. F_{ST} pairwise comparisons (Weir & Cockerham, 1984) for the barnacle and copepod data sets.

Pairwise comparisons						
a.						
Ceratoconcha domingensis	Inner La Parguera	La Parguera Shelf Edge	Guanica Shelf Edge	Vieques	Outer La Parguera	Desecheo
Inner La Parguera	0					
La Parguera Shelf Edge	−0.0029	0				
Guánica Shelf Edge	0.01325	−0.00284	0			
Vieques	−0.01573	−0.03396	0.00693	0		
Outer La Parguera	−0.01247	−0.01096	−0.0167	−0.00138	0	
Desecheo	0.07196*	0.04962	0.01411	0.02239	0.02281	0
b.						
Laophontella armata	La Parguera shallow	El Seco Deep	Curacao Shallow	St. John Intermediate		
La Parguera Shallow	0					
El Seco Mesophotic	0.30298	0				
Curaçao Shallow	0.41243*	0.30967	0			
St. John Mesophotic	0.24076	0.48763	0.48763*	0		

*Represents significant comparisons with a *P*-value ≤0.05.

(included: 0.3097, Table 2; excluded: 0.2804). Sequence divergence values for the COI gene between El Seco and Curaçao with the four divergent sequences excluded ranged from 0.2–1.6%, whereas genetic distance between the divergent sequences and all other sequences ranged between 5.1–7%. Furthermore, AMOVA analyses including all sample localities (El Seco, La Parguera, St. John, Curaçao) were run twice, including ($\Phi_{ST} = 0.2999$) and excluding ($\Phi_{ST} = 0.3883$) the divergent sequences (Table 3).

DISCUSSION

As dramatic phase shifts continue in most marine ecosystems, the risk of losing undocumented marine biodiversity, as well as altering natural gene flow patterns when creating fragmented habitats increases (Plaisance *et al.*, 2011). Gene flow is a complicated process and the result of pre- and post-zygotic factors relating to the species in question and environment where they reproduce (Cowen & Sponaugle, 2009). For instance, the scleractinian coral *Agaricia lamarcki*, has pronounced gene flow between shallow and mesophotic colonies in south-west Puerto Rico (Hammerman *et al.*, 2017). Although, the *Symbiodinium* populations within *A. lamarcki* contain depth-differentiated clades, with specific clade profiles from either shallow or mesophotic depths (Bongaerts *et al.*, 2013; Lucas *et al.*, 2016). Despite insights into demographic properties of corals and their relationship to endosymbionts, relatively little knowledge is available pertaining to speciation and gene flow of coral-associated fauna. By taking a more holistic approach to community-level population structure, a better handle of gene flow can be surmised and it can be

Table 3. AMOVA results for both the barnacle sample set (a) and copepod sample set including (b) and excluding (c) outlier haplotypes.

AMOVA results				
Source of variation	**df**	**Sum of squares**	**Variance components**	**Percentage of variation**
a.				
Ceratoconcha domingensis				
Among populations	5	15.43	0.01295 Va	0.44
Within populations	87	252.324	2.90028 Vb	99.56
Total	92	267.75	2.91322	
Fixation index	F_{ST}: 0.00444			
$P = 0.33376 \pm 0.00484$				
b.				
Laophontella armata (divergent sequences included)				
Among populations	3	21.875	0.61908 Va	30
Within populations	50	72.239	1.44479 Vb	70
Total	53	94.114	2.064	
Fixation index	F_{ST}: 0.29996			
$P = 0.0$				
c.				
Laophontella armata (divergent sequences excluded)				
Among populations	3	5.322	0.1665 Va	38.83
Within populations	46	12.065	0.26229 Vb	61.17
Total	49	17.388	0.42879	
Fixation index	F_{ST}: 0.38831			
$P = 0.0016 \pm 0.00042$				

determined how genetically interconnected coral reefs are with one another and how that leads to both patterns of panmixia and genetic structure. Studies with single species can fail to identify dispersal barriers, for instance the concomitant use of 27 species from different trophic levels indicated the presence of four phylogeographic breaks within the Hawaiian Archipelago (Toonen *et al.*, 2011). A multispecies approach is a more informative and unbiased approach to estimate patterns of connectivity that characterize the whole community (Toonen *et al.*, 2011).

The COI haplotype network and AMOVA analysis of the obligate coral barnacle, *Ceratoconcha domingensis* indicated no geographic partition between La Parguera and Guánica (Figure 2; Tables 2 and 3). Geographically, sample sites are within ~20 km of one another and both La Parguera and Guánica contain connected and continuous reef habitat. Within this continuous reef network, *Agaricia lamarcki* exhibits genetic panmixia (Hammerman *et al.*, 2017) as its obligate barnacle symbiont *C. domingensis*. The high abundance and the plate morphology of *A. lamarcki* are especially conducive for barnacle nauplius settlement (Liu *et al.*, 2017). Predominant westerly driven currents could uniformly transport larvae across south-west Puerto Rico and further aid in propagule retention, allowing for recruitment of coral associated barnacles in a stepping stone fashion (Appeldoorn *et al.*, 1994; Zitello *et al.* 2008). The excess of rare barnacle sequences in all locations is consistent with the scenario of population growth as indicated by the negative values of Fu's *Fs* (Table 1). The demographic history of many marine species is characterized by population expansions (Delrieu-Trottin *et al.*, 2017).

Future investigations should sample Pyrgomatidae barnacles from more species within the *Agaricia* genus as well as other coral hosts (Young & Christoffersen, 1984; Chen *et al.*, 2012). By sampling across all the agariciid corals and across a wider geographic setting, a better handle on biodiversity and host preference can be obtained and will allow for comparative inferences into gene flow and cryptic speciation. The utility of DNA barcoding for elucidating such phenomena has already been proven (Neigel *et al.*, 2007; Plaisance *et al.*, 2009; Raupach & Radulovici, 2015). Specifically, COI barcoding with *Millepora*-associated barnacles (*Wanella* spp.) revealed five distinct clades within sampling sites in Taiwan, corresponding to differences in shell and opercula plate morphology (Tsang *et al.*, 2009). The distinct clades indicated the presence of cryptic species with a significant difference in preference for host growth form between encrusting and branch-forming *Millepora*. A similar trend may persist for agariciid-associated barnacles and warrants further investigation.

High levels of population structure were estimated for the harpacticoid copepod, *Laophontella armata* between the mesophotic reef in El Seco off the eastern coast of Puerto Rico and from a shallow mangrove habitat in Curaçao (Tables 2 and 3). This copepod species is considered a habitat generalist since it inhabits both mangrove and mesophotic habitats. However, shared haplotypes (Haplotype 2 and 6) were identified between the two strikingly different environments (Figure 3). This was unexpected due to the obligatory benthic life history of *L. armata*, the shear geographic distance between the two localities (770 km – straight line; 1790 km – contour of the Caribbean plate), and expectations from previous studies on the population structure of harpacticoid copepods (Burton *et al.*, 1979; Burton, 1998; Schizas *et al.*, 1999; Gregg *et al.*, 2010; Handschumacher *et al.*, 2010). Most studies on harpacticoid copepods reveal population structure on small and large geographic scales (e.g. Schizas *et al.*, 1999, 2002; Edmands, 2001; Denis *et al.*, 2009; Willett & Ladner, 2009; Gregg *et al.*, 2010). Both mud-dwelling harpacticoid copepods, such as *Microarthridion littorale*, and rock pool copepods such as *Tigriopus* spp. exhibit population subdivision along saltmarsh estuaries (Schizas *et al.*, 1999) and rock pools (Handschumacher *et al.*, 2010), respectively. In a more extreme setting, research on deep-sea hydrothermal vent-associated siphonostomatoid copepods showed high levels of gene flow and genotypic diversity across several ocean basins suggesting copepod populations could be large and continually undergoing population growth (Gollner *et al.*, 2016). Sometimes, species with limited dispersal can be more widespread than those with extensive plankton dispersal, since restricted dispersal can help maintain a population at high densities after rare, long-distance transport events (Johannesson, 1988). These rare transport events could explain the shared haplotypes observed between *Laophontella* subpopulations from El Seco (Puerto Rico), Vieques and Curaçao.

The three divergent haplotypes (Hap 21, 22 and 23) from El Seco were consistently BLASTed as harpacticoid copepod sequences, and they may represent several morphologically similar species. The only species of *Laophontella* recorded from the tropical Atlantic/Caribbean (Bermuda (Willey, 1935) and Bahamas (Geddes, 1968)) is *Laophontella armata*. The morphology of *Laophontella* is very distinct with large cuticular spines and is almost impossible even for a novice to mistaken their identity. All specimens were sorted and few were dissected by experienced copepodologists, therefore if there are cryptic species, they share the same general body morphology with *Laophontella* and belong to this genus. Alternatively, these haplotypes represent uncommon deeply divergent lineages within *Laophontella*, at least in El Seco, Vieques. This discovery could also indicate a vast underestimation of mesophotic reef-associated copepod biodiversity and more generally meiofauna and small macrofauna. It has been suggested that MCEs represent a hotspot of biodiversity with dozens of new species of macroalgae (e.g. Ballantine & Ruiz, 2010), fish (e.g. Pyle & Kosaki, 2016), crustaceans (e.g. Schizas *et al.*, 2015) and mites (e.g. Pešić *et al.*, 2014) being described in recent years. These taxonomic studies highlight the importance of MCEs and the need for these deeper habitats to be protected with similar management policies as the shallow water coral reefs.

The extreme case of shared haplotypes in copepods between the El Seco mesophotic reef off the east coast of Puerto Rico and a shallow mangrove habitat in Curaçao show how interconnected seemingly disjunct populations of crustaceans can be and reveal a shared genetic and biological diversity between shallow and mesophotic habitats. This is an important issue in the central question of whether MCEs are refuge habitats for all or only a few species (Bongaerts *et al.*, 2017) since shared genetic backgrounds are indicative of species with high connectivity. Further research should focus on sampling more mesophotic reef-associated fauna to discern habitat usage, gene flow, host preference and specificity. This is especially important given the remarkable biodiversity encountered in both shallow and mesophotic reefs. More studies are needed to further elucidate the shared

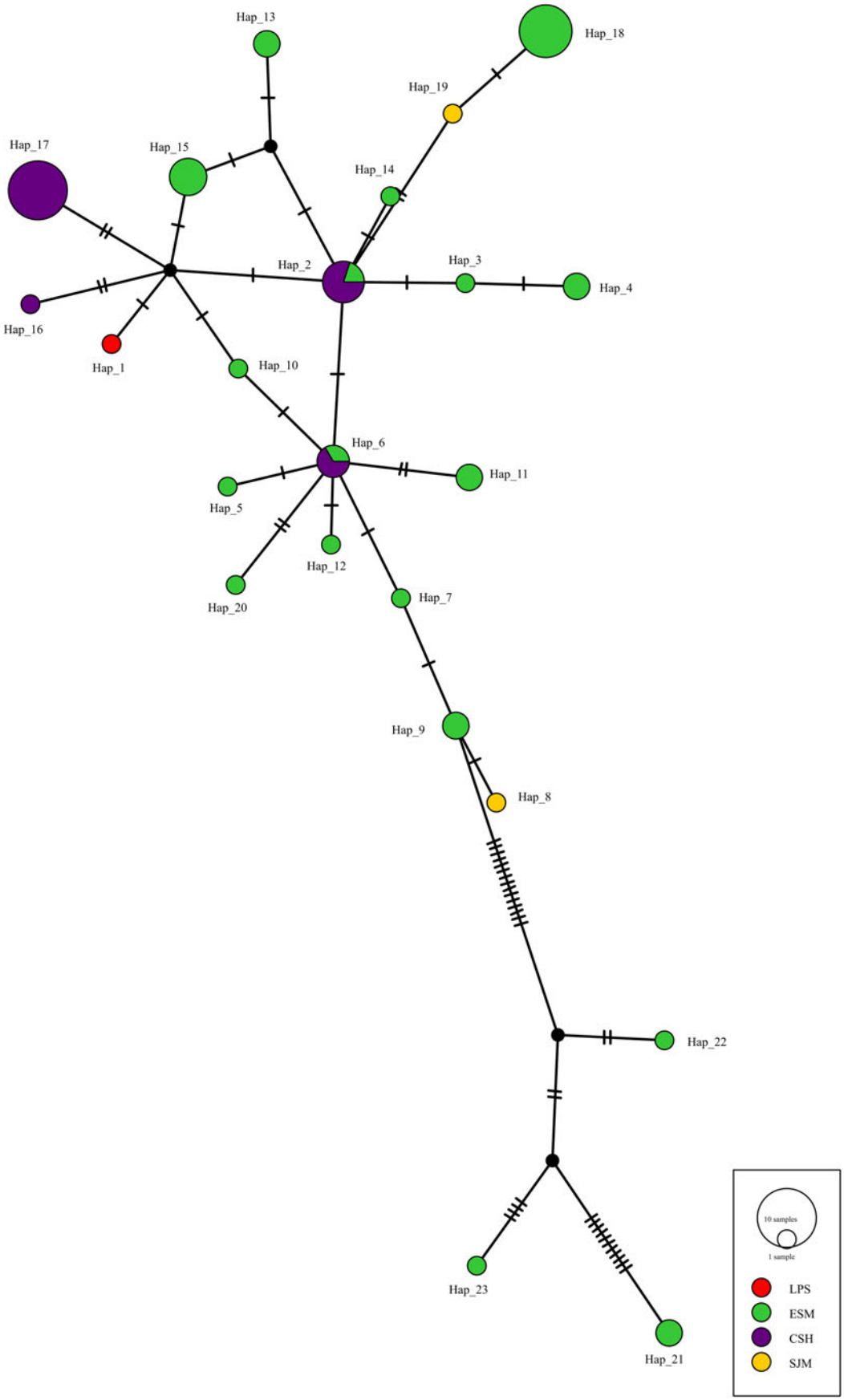

Fig. 3. Median-joining network based on *Laophontella armata* COI haplotypes generated in this study. The network was estimated using the median-joining algorithm in PoPArt with epsilon = 0. Each circle represents a different haplotype and the size of a circle correlates with number of individuals belonging to that given haplotype. Colours indicate the geographic origin of sequences as follows: i. La Parguera Shelf Edge (LPS), red; ii. El Seco Mesophotic (ESM), green; iii. Curaçao Shallow, purple; iv. St. John Mesophotic (SJM), yellow.

meio- and macrofauna habitats and possible routes of genetic exchange. In conclusion, panmixia estimated between the coral-inhabiting barnacle and the shared haplotypes found between the neritic and mesophotic locations for the harpacticoid copepod, highlights the genetic interplay between shallow and deeper habitats and the significance of using multiple species to decipher population connectivity at the community level.

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