MOLECULAR ECOLOGY
Molecular Ecology (2015) **24**, 1447–1466 doi: 10.1111/mec.13134

Population structure and dispersal of the coral-excavating sponge *Cliona delitrix*

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Abstract

Some excavating sponges of the genus *Cliona* compete with live reef corals, often killing and bioeroding entire colonies. Important aspects affecting distribution of these species, such as dispersal capability and population structure, remain largely unknown. Thus, the aim of this study was to determine levels of genetic connectivity and dispersal of *Cliona delitrix* across the Greater Caribbean (Caribbean Sea, Bahamas and Florida), to understand current patterns and possible future trends in their distribution and effects on coral reefs. Using ten species-specific microsatellite markers, we found high levels of genetic differentiation between six genetically distinct populations: one in the Atlantic (Florida-Bahamas), one specific to Florida and four in the South Caribbean Sea. In Florida, two independent breeding populations are likely separated by depth. Gene flow and ecological dispersal occur among other populations in the Florida reef tract, and between some Florida locations and the Bahamas. Similarly, gene flow occurs between populations in the South Caribbean Sea, but appears restricted between the Caribbean Sea and the Atlantic (Florida-Bahamas). Dispersal of *C. delitrix* was farther than expected for a marine sponge and favoured in areas where currents are strong enough to transport sponge eggs or larvae over longer distances. Our results support the influence of ocean current patterns on genetic connectivity, and constitute a baseline to monitor future *C. delitrix* trends under climate change.

Keywords: Caribbean Sea, connectivity, coral death, marine sponge, population genetics

Received 7 October 2014; revision received 13 February 2015; accepted 20 February 2015

Introduction

Sponges are the dominant habitat-forming animals in Caribbean coral reefs (Pawlik 2011). Their diversity is higher than all coral groups combined, and their biomass (weight, volume) can exceed corals and algae (Rützler 1978; Díaz & Rützler 2001). The increase in sponges over corals in Caribbean reefs is attributed to factors such as pollution, overfishing and coral mortality (Ward-Paige *et al.* 2005; Chaves-Fonnegra 2014; Loh & Pawlik 2014). Some sponge species have become abundant in areas where sewage pollution occurs (Rose & Risk 1985; Holmes 1997; Ward-Paige *et al.* 2005). Simultaneously, overfishing has removed sponge predators, which can increase the competition for space between faster-growing palatable sponges and reef-building corals (Loh & Pawlik 2014). Higher coral mortality has also opened more habitat for sponge larvae to attach to dead coral skeletons (Chaves-Fonnegra 2014).

Many sponges have successful survival strategies to compete for space and modify coral reefs through bioerosion (Rützler 1975; Díaz & Rützler 2001; Chaves-Fonnegra & Zea 2007), calcification or cementation (Wulff & Buss 1979; Wulff 1984). Coral-excavating sponges can bioerode entire coral colonies through chemical and physical mechanisms (Pomponi 1979; Zundelevich *et al.* 2007), and they can increase their boring rates at lower pH (Wisshak *et al.* 2012; Stubler *et al.* 2014) and grow faster in warmer temperatures (Cortés *et al.* 1984;

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Rützler 2002), abilities that may favour them during climate change scenarios (Carballo *et al.* 2013). These sponges have become more abundant during the past few decades in the Caribbean Sea and Florida (López-Victoria & Zea 2004; Ward-Paige *et al.* 2005; Chaves-Fonnegra *et al.* 2007). Specifically, *Cliona delitrix* is one of the most destructive species, with its ability to kill coral tissue (Chaves-Fonnegra & Zea 2007) and excavate 10–12 cm inside coral skeletons, spreading laterally at mean rates of ~1.5 cm/year (Chaves-Fonnegra & Zea 2011). This species, currently, is one of the most conspicuous in the Florida Keys as well as in other areas in the Caribbean Sea (Rose & Risk 1985; Ward-Paige *et al.* 2005; Chaves-Fonnegra *et al.* 2007).

Understanding population dynamics of marine sponges will be vital to potentially predict future changes at a reef level, especially for excavating sponges, which can have a strong impact on reef-building corals. Important aspects affecting excavating sponges' distribution, such as dispersal capability and population genetics structure, remain largely unknown. The only population genetics study on excavating sponges estimates that the dispersal distance of *C. delitrix* reaches beyond 10–100 m and appeared to be sexual through larvae, rather than asexually through fragmentation or clonality (Zilberberg *et al.* 2006). Sexual reproduction for this species has recently been characterized (Chaves-Fonnegra 2014).

A prevailing view is that Poriferan species exhibit low dispersal; most larvae remain in the water column for a short period of time, minutes to few days, and usually <2 weeks (Bergquist & Sinclair 1968, 1973; Ilan & Loya 1990; Meroz & Ilan 1995; Mariani *et al.* 2000; Maldonado 2006). The larvae of *Cliona* sponges are defined as clavablastula, as they develop from a hollow embryo that later fills with maternal cells (Maldonado & Bergquist 2002; Maldonado 2006), also with low dispersal (Mariani *et al.* 2000, 2006). Observations of *Cliona viridis* indicate that its larvae are small (300 μm length × 100 μm width) and weak swimmers that can stay in the water column for less than 10 days (Mariani *et al.* 2000, 2006). In the laboratory, and inside beakers, larvae from *C. celata* can swim continuously on the surface for only 20–30 h with no apparent reaction to daylight or darkness; then, they start to move to the substrate for approximately the same time they spend at surface, until attaching (Warburton 1966).

Early population genetics studies in the phylum Porifera used allozymes to determine phylogenetic- and population-level differences in allele frequencies and contributed to the discovery of cryptic species (Solé-Cava & Thorpe 1986; Sara *et al.* 1988). Mitochondrial DNA (mtDNA), specifically the commonly used Folmer's region 5′ end of the COI gene (Folmer *et al.* 1994), shows relatively low genetic variation within sponge species (Wörheide *et al.* 2005), with exceptions (Duran & Rützler 2006; Debiasse *et al.* 2010). Other partitions of the mtDNA are also useful for phylogeographic analyses (Erpenbeck *et al.* 2006; Lopez-Legentil & Pawlik 2009; Rua *et al.* 2011).

Nuclear DNA microsatellite loci are more informative and variable markers for population genetics of many organisms, including marine sponges (Wörheide *et al.* 2005). Microsatellite loci have been identified in five demosponge species, *Halichondria panacea* (Knowlton *et al.* 2003), *Crambe crambe* (Duran *et al.* 2004), *Scopalina lophyropoda* (Blanquer *et al.* 2005), *Xestospongia muta* (Richards 2010) and *Spongia officinalis* (Dailianis *et al.* 2011), and one calcareous sponge, *Paraleucilla magna* (Guardiola *et al.* 2012). These studies have been useful for comparisons within the Mediterranean Sea (Dailianis *et al.* 2011; Guardiola *et al.* 2012), between Mediterranean and Atlantic populations (Duran *et al.* 2004), and also at a much finer scale, between populations separated by only 10–50 m (Guardiola *et al.* 2012) and~ 25–100 m (Blanquer *et al.* 2009) and between individuals separated 0–7 m from each other (Calderón *et al.* 2007).

With evidence that excavating sponges continue to increase in abundance on Caribbean coral reefs (Cortés *et al.* 1984; Rose & Risk 1985; Rützler 2002; Ward-Paige *et al.* 2005; Chaves-Fonnegra *et al.* 2007), our goal in this study was to examine genetic connectivity and dispersal of *Cliona delitrix* across the Greater Caribbean using microsatellite DNA markers.

Methods

Sample collection and DNA extraction

A total of 540 samples were collected from coral reefs in 12 locations within the Great Caribbean (see Table 1, Fig. 1). Maximum depth of all locations was obtained in situ (Table 1), and shorter distance to the coast of the seven sampling locations in Florida (USA) was measured in Google Earth (http://www.google.com/intl/en_uk/earth/) using sites coordinates (see Appendix S1, Supporting information). All samples were placed in 15-mL falcon tubes and preserved in 95% ethanol at −20 °C; three changes in 95% ethanol after 1 h, 24 h and 3 days were made to eliminate some secondary metabolites before DNA extractions, following PORTOL recommendations (https://www.portol.org/node/24). Genomic DNA was extracted from 50 mg of *Cliona delitrix* tissue (mostly from oscula to avoid calcium carbonate) using the DNeasy Tissue Kit (Qiagen Inc.). During the incubation step with proteinase K, samples were agitated for 12 h at 60 °C at 150 rpm in a Sheldon VWR 1575 Incubator. One modification to the kit was made:

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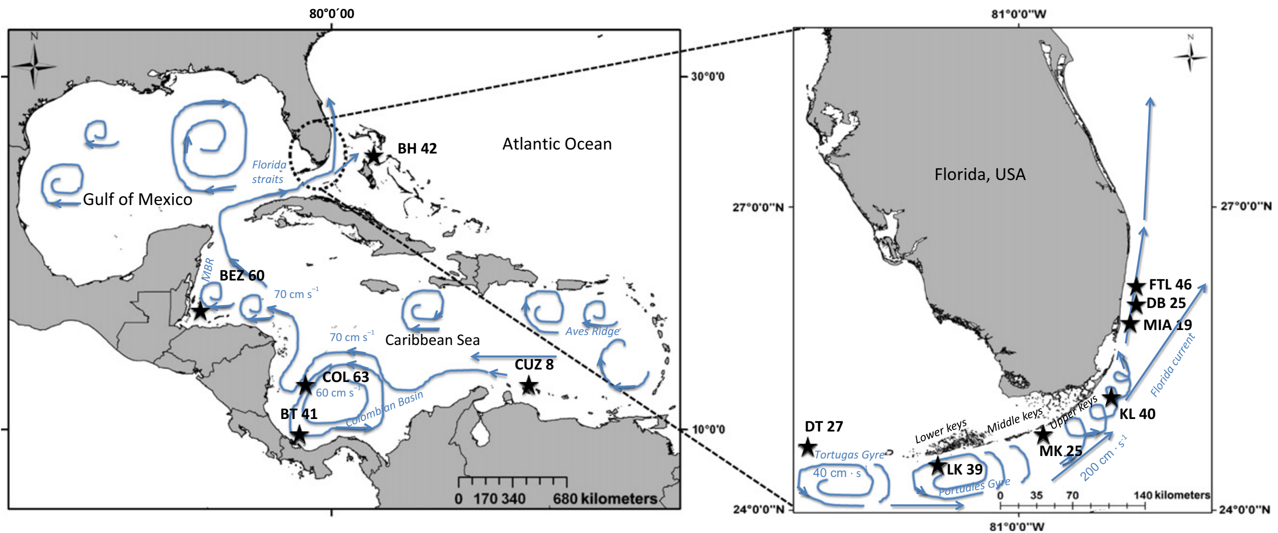

Fig. 1 Area of study (Caribbean Sea and Atlantic) with sampled locations marked as black stars. BH: Bahamas (Various reefs); FTL: Florida (Fort Lauderdale); DB: Florida (Dania Beach); MIA: Florida (Miami); KL: Florida (Key Largo); MK: Florida (Middle Keys); LK: Florida (Looe Key); DT: Florida (Dry Tortugas); BEZ: Belize (Carrie Bow Cay); COL: Colombia (San Andrés Island); BT: Panama (Bocas del Toro); CUZ: Curaçao (Carmabi). Numbers in black represent the total analysed samples for each location. Blue lines represent the main ocean currents and gyres, and the speed of some currents is included (adapted from Lee *et al.* 1994; Lee & Williams 1999; Andrade & Barton 2000).

after incubation in proteinase K, instead of vortexing the sample (tissue + calcium carbonate + spicules), we only took the supernatant from each sample, avoiding mixing with any residue at the bottom of tubes. This eliminated spicules and calcium carbonate that could clog the filter columns in subsequent steps.

De novo development of Cliona delitrix-specific microsatellite markers

Genomic DNA (gDNA) from one individual (originally from Carry Bow Cay, Belize) was used to isolate microsatellites markers using the protocol of Glenn & Schable (2005). The gDNA was digested using RsaI and XmnI restriction enzymes, and following digestion, the SuperSNX24 linkers were ligated onto the ends of gDNA fragments. These linkers are sites for primers in subsequent polymerase chain reactions (PCRs). To obtain fragments of gDNA enriched with microsatellite sequences, we hybridized twelve biotinylated probes to the cut gDNA in three separate reactions: one ($ACAT_8$, $AAGT_8$, $AAAT_8$, $AGAT_8$, $AACT_8$), two (TG_{12}, AG_{12}) and three ($ACTC_6$, $ACAG_6$, $AAAC_6$, $AAAG_8$ and $ACCT_6$). The gDNA–biotinylated probe complex was added to magnetic beads coated with streptavidin (Invitrogen). This complex was washed with two hybridization buffers, twice with 12 X saline–sodium citrate (SSC) + 0.2% sodium dodecyl sulphate (SDS), and four times with 6 X SSC + 0.1% SDS incubating at 53 °C for 1 min each for the final two washes. The probe–DNA complex was denaturated at 95 °C, and the resultant enriched fragments were precipitated with 3 M sodium acetate and 95% ethanol.

To increase the amount of enriched fragments, a 'recovery' PCR was performed in a 25 μL reaction containing 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.5 mM $MgCl_2$, 2.5 μL of 10X bovine serum albumin (BSA), 0.16 mM of each dNTP, 10X BSA, 0.52 mM of the SuperSNX24 forward primer, 1 U *Taq* DNA polymerase and approximately 25 ng enriched gDNA fragments. Thermal cycling was performed in a Bio-Rad DYAD, as follows: 95 °C for 2 min; followed by 25 cycles of 95 °C for 20 s, 60 °C for 20 s and 72 °C for 90 s; and a final elongation step of 72 °C for 30 min. PCR products were inserted into vectors using the TOPO-TA Cloning®kit (Invitrogen), and vectors were incorporated into *Escherichia coli* competent cells and incubated overnight at 37 °C on ampicillin LB Agar plates. Bacterial colonies containing a recombinant vector (i.e. white colonies) were isolated, and their gDNA inserts were amplified in a 25 μL PCR containing 1 X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.5 mM $MgCl_2$, 0.12 mM of each dNTP, 10 X BSA, 0.25 mM of the M13 primers and 1 U *Taq* DNA polymerase. Thermal cycling conditions were 95 °C for 7 min, followed by 35 cycles of 95 °C for 20 s, 50 °C for 20 s and 72 °C for 90 s. Resulting PCR products were cleaned with ExoSAP-IT (Affymetrix, Inc., Santa Clara,

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Table 1 Number (*n*) of *Cliona delitrix* samples collected, amplified and analysed from the Greater Caribbean (including Caribbean Sea, Bahamas and Florida). Morph – external morphology of the sponges when comparing individuals of similar size; Collect – total number of samples collected in each specific geographic area; Amp – total number of samples amplified at least with seven of the 10 microsatellites; Analys – total number of samples analysed after eliminating clones

Geographic region	Location	Specific area	Morph	Label	Depth (m)	Distance to coast (km)	Collect	Amp	Analys
Atlantic Ocean	Bahamas	Various areas (Bimini, Cat Cays, New Providence, Little San Salvador, San Salvador, Inaguas)	B, C	BH	4–18	82.2–605.8	46	43	42
Atlantic Ocean	Florida	Fort Lauderdale (shallow reef)	A	FTL	8	0.5	54	48	46
Atlantic Ocean	Florida	Dania Beach (shallow reef)	A	DB	9	1.4	31	30	25
Atlantic Ocean	Florida	Miami (shallow reef)	A	MIA	6	1.5	19	19	19
Atlantic Ocean	Florida	Key Largo (North of North Dry Rocks – Conch Wall)	A	KL	10–20	7.2	50	47	40
Atlantic Ocean	Florida	Middle Keys (Marathon Thor patch –Long Key foot mound reef)	A	MK	8	3.4	31	30	25
Atlantic Ocean	Florida	Looe Key (East, outside sanctuary)	A	LK	11	8.2	53	41	39
Atlantic Ocean	Florida	Dry Tortugas (Texas Rock – 8 Fathom – The Maze)	A	DT	14–22	121	34	33	27
Southwest Caribbean	Belize	Carrie Bow Cay (Patch and fore reefs)	B	BEZ	8–25	—	77	72	60
Southwest Caribbean	Colombia	San Andres Island (Wildlife)	B	COL	1–9	—	70	70	63
Southwest Caribbean	Panama	Bocas del Toro (Punta Caracol, Adrianas)	B	BT	6–10	—	65	54	41
Southeast Caribbean	Curaçao	Reef in front Carmabi Research Station	B, C	CUZ	5–15	—	10	8	8
					Total		540	495	435

A: encrusting growth form, oscules tend to be taller than wider and reddish; B: encrusting growth form, oscules tend to be wider than taller and yellowish with internal divisions; C: papillated growth form, smaller oscules tend to be yellow.

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CA, USA) and sequenced using the BigDye® TERMINATOR v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing reactions were precipitated with ethanol and 125 mM EDTA, and run on an ABI 3730 DNA Analyzer (Applied Biosystems).

Primers pairs were developed for 32 core microsatellites repeats using PRIMER 3 v.4.0. on the Web (Koressaar & Remm 2007; Untergrasser *et al.* 2012). Ten of these loci amplified reliably and showed evidence of polymorphism. These loci were used to genotype 495 individuals of the coral-excavating sponge *Cliona delitrix,* and their cross-amplification utility was examined with eight individuals of *C. laticavicola*. Amplification of microsatellites was performed in total PCR volumes of 25 μL. Two of the ten loci amplified better with Promega Hot Start enzyme, and the other eight with Qiagen Hot Start enzyme (Table S1, Supporting information). The PCR with Promega enzyme contained 5 μL of 5X PCR buffer, 3 μL of 25 mM $MgCl_2$, 1.2 μL of 10 mM dNTPs mix, 1 μL of 10 μM fluorescently labelled universal M13 primer (5′-TGTAAAACGACGGCCAGT-3′) (Schuelke 2000), 0.5 μL of 10 μM species-specific forward primer with a 5′ M13 tail (Schuelke 2000), 1 μL of the 10 μM reverse species-specific primer, 8.05 μL of dH_2O, 5 μL of DNA template (0.1–2 ng/μL which was diluted from stock gDNA; 1:100 or 1:1000) and 0.125 μL of 5U Hot Start Taq DNA Polymerase (Promega). The Qiagen PCR contained 2.5 μL of 10X PCR buffer, 1.5 μL of 25 mM $MgCl_2$, 1.2 μL of 10 mM dNTPs mix, 1 μL of 10 μM fluorescently labelled universal M13 primer (5′-TGTAAAACGACGGCCAGT-3′) (Schuelke 2000), 0.5 μL of 10 μM species-specific forward primer attached with a 5′ M13 tail (Schuelke 2000), 1 μL of the 10 μM reverse species-specific primer, 12.175 μL of dH_2O, 5 μL of DNA template (0.1–2 ng/μL which was diluted from stock gDNA) and 0.125 μL of 2.5U Hot Start Taq DNA Polymerase (Qiagen). PCR was performed in a gradient thermal cycler (Bio-RadC1000) as follows, for Promega: 94 °C for 4 min; followed by 30 cycles of 94 °C for 15 s, 15 s at the primer annealing temperature (Table S1, Supporting information) and 72 °C for 45 s; followed by eight cycles of 94 °C for 15 s, 53 °C for 15 s and 72 °C for 45 s; and a final elongation step of 72 °C for 10 min. For Qiagen: 95 °C for 5 min; followed by 34 cycles of 94 °C for 1 min, 30 s at the primer annealing temperature (Table S1, Supporting information) and 72 °C for 1 min; and a final elongation step of 72 °C for 10 min. Products were visualized on a 3730 DNA Analyzer (Applied Biosystems). Alleles were sized using the internal standard GeneScan 500 LIZ (Applied Biosystems), and electropherograms were analysed using GENEMAPPER version 3.7 (Applied Biosystems).

Summary statistics

Number of alleles, F_{IS}, F_{ST} and levels of observed (H_O) and expected (H_E) heterozygosity across microsatellite loci were obtained with the Microsatellite toolkit (Park 2001) and FSTAT 2.9.3.2 (Goudet 1995). Tests for linkage equilibrium (LE) and deviations from Hardy–Weinberg (HWE) for each locus and location were estimated using unbiased exact tests (Markov chain method) with 10 000 dememorizations, 1000 batches and 10 000 iterations per batch (Guo & Thompson 1992), as implemented in GENEPOP v.4.2 on the Web (Raymond & Rousset 1995b; Rousset 2008). Significance levels (≤0.05) were adjusted using the sequential Bonferroni correction for multiple pairwise testing (Rice 1989). The linkage disequilibrium correlation coefficient (r_{LD}) was calculated in LinkDos on the Web program (Garnier-Gere & Dillmann 1992) as an index to decide whether loci should be included in the Bayesian Analysis in STRUCTURE (Kaeuffer *et al.* 2007).

The number of private alleles per population was estimated using GENALEX 6.5 (Peakall & Smouse 2012). The frequency of null alleles was estimated using the expectation maximization (EM) algorithm (Dempster *et al.* 1977) as implemented in FREENA (Chapuis & Estoup 2007).

Considering that null alleles have the potential to increase F_{ST} values (Chapuis & Estoup 2007), the locus with highest null allele frequency was systematically removed from the data set, and then, the standard F_{ST}, corrected F_{ST} for null alleles, F_{IS} and mean of null allele frequencies per locus (10 to 1) were recalculated and compared.

Population differentiation

Four different statistical tests were performed to test for population differentiation: pairwise Fisher's exact (population level), multivariate statistical analysis (population and individual levels), Bayesian algorithm (individual level) and iterative reallocation method (individual level). We determined the number of populations based on concordance between tests. Analyses at the individual level were performed with all the sampled locations in the Greater Caribbean, only Atlantic locations (Florida and Bahamas) and only South Caribbean locations (Belize, Colombia, Panamá and Curaçao). To be conservative with our estimates of differentiation, we ran the analyses that assume linkage equilibrium as a priori information (Fisher's exact test and Bayesian algorithm) with and without linked loci. Multivariate analysis and iterative reallocation statistical tests were run with all loci, as both methods do not assume linkage equilibrium.

Pairwise Fisher's exact test – population level. To evaluate population differentiation, Fisher's exact tests (Raymond

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& Rousset 1995a) were performed as implemented in FSTAT version 2.9.3.2 (Goudet 1995). This test uses the sampling location as a priori and was performed not assuming HWE within samples, set for 1000 permutations, and with $\alpha = 0.05$ as a baseline to make the Bonferroni correction, following the ad hoc procedure proposed by Waples & Gaggiotti (2006). To test the possible effect that linked loci could have on population differentiation, the linked loci were removed and the analyses were repeated.

Multivariate statistical analysis – population and individual levels. A principal coordinates analysis (PCoA) was conducted at both population and individual levels using the GENALEX 6. Software (Peakall & Smouse 2006). PCoA is a multivariate statistical analysis that uses summarized genetic distances between individual multilocus genotypes to cluster individuals relative to each other in a multidimensional space, without the assumptions of HWE and LE. For the PCoA, two types of genetic distance matrices were calculated: a pairwise, individual-by-individual ($N \times N$) matrix and a population-level pairwise F_{ST} matrix. Also, a geographic distance matrix was calculated based on the GPS coordinates after conversion to decimal degrees. Both genetic and geographic matrices were used to perform the PCoA.

Bayesian algorithm – individual level. To determine the most likely number of genetically discrete populations [Ln Pr ($X|K$)], we used the Bayesian algorithm as implemented in STRUCTURE v2.31 (Pritchard *et al.* 2000). Two different admixture models were used, one without a priori sampling location information (admixture) and one including the sampling location for each individual (admixture + *locprior*) (Hubisz *et al.* 2009). These two models were run with 10 and six microsatellites (after eliminating linked loci), and the parameters included 200 000 burn-in iterations, in ten replicate sets, followed by 200 000 Markov chain Monte Carlo (MCMC) repetitions. The number of genetically homologous groups (K) was determined using the ad hoc statistics ΔK (Evanno *et al.* 2005) implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2012) and visualized using CLUMPP (Jakobsson & Rosenberg 2007) and DISTRUCT (Rosenberg 2004). STRUCTURE was first run with the total data set (435 individuals) and $K = 1$–20 to determine strong trends for population subdivision at the Greater Caribbean level and then, based on initial results, only with the genotypes corresponding to Florida–Bahamas (263 individuals, $K = 1$–15) and South Caribbean (172 individuals, $K = 1$–10).

Iterative reallocation method – individual level. We also used the iterative reallocation method of Paetkau (Paetkau *et al.* 1995), which uses multilocus maximum likelihood and the 'leave-one-out' procedure implemented in FLOCK 3.1. (Duchesne & Turgeon 2012). This method is performed with sampling location information, but does not make use of HWE or LE as a priori information (Duchesne & Turgeon 2012). The partition of the sampled specimens was done in a random mode, with 20 iterations and 50 runs for each k (clusters) assessed ($k = 2$–10) for allocations at the Greater Caribbean, and based on initial results, we repeated the analysis including only individuals for Bahamas–Florida and only for South Caribbean Sea. The stopping and estimation rules were used to determine the number of clusters (K) following Duchesne & Turgeon (2012).

Dispersal patterns

Genetic isolation by geographic distance – population level. Isolation by geographic distance was evaluated (using both linked and unlinked loci) between the pairwise $F_{ST}/(1–F_{ST})$ values and the logarithm of the geographic distances (latitude/longitude in decimal degrees) between locations (Rousset 1997). The variation of $F_{ST}/(1–F_{ST})$ with distance was used as it gives the most easily interpretable information (see Rousset 1997). To test for significant correlation, a Mantel test with 9999 permutations was used as implemented in GENALEX 6.5. (Peakall & Smouse 2012). The test was performed at the Greater Caribbean including all locations. Then, considering results, at this level, we repeated the test only for Florida and Bahamas and only for the South Caribbean locations.

Detection of first-generation migrants – individual level. To evaluate the extent of contemporary (ecological timescale) dispersal of *Cliona delitrix* among geographic locations, the number of first-generation immigrants into each location was inferred using the Bayesian assignment method by Rannala & Mountain (1997) implemented in GENECLASS2 (Piry *et al.* 2004). This method computes the probability that the multilocus genotype of each individual will be encountered in a given population and is a more appropriate test when population differentiation is low and loci deviate from Hardy–Weinberg equilibrium (Rannala & Mountain 1997). For the analysis, the database included only individuals that amplified with all 10 loci (total: 348 individuals). Given that this analysis assumes linkage equilibrium, we used all 10 loci and also repeated the analysis using only 6 loci, after eliminating the linked ones. The statistical criterion computed for likelihood estimation was L_{home}, as it was considered that some source populations for immigrants were probably not sampled (Piry *et al.* 2004). For the probability of computation, we combined the Monte Carlo resampling procedure of Paetkau *et al.*

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(2004) with the likelihood criteria of Rannala & Mountain (1997) with 10 000 simulated individuals and $\alpha = 0.01$ and $\alpha = 0.05$. Specifically for *Cliona delitrix*, detachment of whole individuals rarely occurs (personal observation). Therefore, we consider that migrants correspond to the dispersal of eggs or larvae.

Results

De novo development of Cliona delitrix microsatellite markers

Of 32 loci we tested, 22 did not amplify or showed an excess of peaks in electropherograms, making them difficult to genotype. Ten final primers (Table S1, Supporting information) yielded scorable peaks and were used to genotype 495 individuals. These loci were also useful for cross-amplification in *Cliona laticavicola* (Table S2, Supporting information).

Summary statistics

From a total of 495 individuals of *Cliona delitrix* genotyped, 60 were found to have identical genotypes (potential clones) and were eliminated from population genetics estimators. The total number of alleles per locus ranged from 5 to 78 (Table S5, Supporting information), and the average alleles per population varied from 5.4 (Curaçao) to 16.3 (Bahamas). Private alleles were present in all studied locations. South Caribbean locations and Bahamas had a greater number (11–36) and higher average frequencies (3.0–14.2%) of private alleles, than any of the Florida locations (1–8 private alleles with average allele frequencies between 1.4% and 2.6%), except for Dania Beach which had only two private alleles with an average frequency of 5.2%.

Four of the 10 loci (Cd14, Cd81, Cd114 and Cd137) showed linkage disequilibrium (LD) in a global analysis. However, linkage between pairs of loci was not consistent across all populations (see Table S3, Supporting information). Thus, the population genetics analyses that assume no LD (Pairwise Fisher's exact test, Bayesian algorithm) were performed with both 10 and 6 loci, after eliminating the four linked loci. The r_{LD} was lower than 0.5 between all loci pairs in which the analysis was possible at a global level suggesting all the loci could be used in the Bayesian cluster analysis (see Table S4, Supporting information) (Kaeuffer *et al.* 2007).

Inbreeding coefficient (F_{IS}) values per population were positive and significant ($P < 0.00042$), except for Curaçao. F_{IS} in Belize was positive, but not significant after Bonferroni correction. This indicates a heterozygote deficit in most locations (Table S5, Supporting information), suggesting nonrandom mating between individuals. The exact test for HWE confirmed these results by showing significant deviations in each location, except Curaçao (Table S5, Supporting information). The average frequency of null alleles for each locus ranged from 0.033 to 0.122 and for each population ranged from 0.022 to 0.089 (Table S5, Supporting information). The F_{ST} corrected values were slightly lower than the standard F_{ST} values, and the difference between both parameters fluctuated between 0.004 and 0.008. F_{IS} values were higher when null allele frequency was higher (using all 10 loci) and decreased when loci with higher null allele frequency were removed. However, the standard F_{ST} and corrected F_{ST} values remained relatively stable until a fifth locus (Cd23) was removed. Then, a decrease of 0.054 units occurred, and both values continued decreasing until all loci, except one, were removed.

Population genetic structure of Cliona delitrix

Pairwise Fisher's exact test – population level. F_{ST} values showed population structure between geographic locations. Using 10 loci, Florida locations (Fort Lauderdale, Dania Beach, Miami, Key Largo, Middle Keys, Looe Key and Dry Tortugas) were genetically different from South Caribbean locations (Belize, Colombia and Panamá) ($P < 0.05$; Table 2). However, Curacao was not different from the Middle Keys location in Florida ($P = 0.2056$), and Bahamas was divergent to all Florida and South Caribbean locations. Inside Florida, genetic similarity was found among Dry Tortugas, Looe Key and Key Largo, and between Looe Key and Dania Beach, Dania Beach and Fort Lauderdale, Fort Lauderdale and Miami, and Miami and the Middle Keys (Table 2). When the four linked loci were removed, similar results than with 10 loci were obtained, with only two exceptions: Fort Lauderdale was genetically different from Dania Beach ($P = 0.0616$), but Dania Beach was similar to the Middle Keys ($P = 0.0545$; see Table 2).

Multivariate statistical analysis – population and individual levels. The PCoA analysis suggested four genetic groups in the total microsatellite data set: (i) Atlantic, (ii) Florida, (iii) South Caribbean and (iv) Curaçao (Fig. 2). Axis 1 explained 30.9% of the variation found in the distance matrix, whereas axis 2 and axis 3 explained 27.3% and 16.7%, respectively. Axis 1 separated the South Caribbean populations (Belize, Bocas del Toro, Colombia and Curaçao) from Atlantic (Bahamas, Key Largo, Looe Key, Dry Tortugas). Axis 2 separated a second group of Florida populations (Dania Beach, Fort Lauderdale, Miami and Middle Keys) together with Curaçao. Axis 3 separated Curaçao from the rest of populations

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Table 2 F_{ST} Values between populations using all 10 loci (top number), or only 6 non-LD loci (bottom number in parenthesis). For exact test after sequential Bonferroni correction (α 0.000758): (*) significant differences between geographic locations, BH: Bahamas (Various reefs); FTL: Florida (Fort Lauderdale); DB: Florida (Dania Beach); MIA: Florida (Miami); KL: Florida (Key Largo); MK: Florida (Middle Keys); LK: Florida (Looe Key); DT: Florida (Dry Tortugas); BEZ: Belize (Carrie Bow Cay); COL: Colombia (San Andrés Island); BT: Panama (Bocas del Toro); CUZ: Curaçao (Carmabi)

	BH	FTL	DB	MIA	KL	MKY	LK	DT	BEZ	COL	BT
FTL	0.1861* (0.1752)*										
DB	0.0805* (0.0824)*	0.0549 (0.0616)*									
MIA	0.192* (0.2004)*	0.0543 (0.0779)	0.079* (0.1054)*								
KL	0.0384* (0.0359)*	0.1689* (0.159)*	0.0526* (0.055)*	0.1797* (0.1951)*							
MKY	0.2211* (0.1791)*	0.0318* (0.0273)	0.0743* (0.0545)	0.0335 (0.0391)	0.2046* (0.1673)*						
LK	0.0469* (0.0511)*	0.1243* (0.1093)*	0.0202 (0.0147)	0.139* (0.1449)*	0.0117 (0.0174)	0.155* (0.1031)*					
DT	0.0399* (0.0382)*	0.1398* (0.121)*	0.0349* (0.0327)*	0.1554* (0.1604)*	0.0083 (0.0127)	0.1705* (0.1206)*	0.0002 (−0.0026)				
BEZ	0.1667* (0.0999)*	0.1669* (0.1961)*	0.1337* (0.1416)*	0.1981* (0.2311)*	0.1725* (0.1285)*	0.1815* (0.2146)*	0.1517* (0.1059)*	0.143* (0.0891)*			
COL	0.222* (0.1284)*	0.2222* (0.2152)*	0.1828* (0.1552)*	0.244* (0.2445)*	0.2176* (0.1534)*	0.2129* (0.2095)*	0.2006* (0.1219)*	0.1985* (0.1093)*	0.1164* (0.0999) *		
BT	0.2503* (0.1204)*	0.2413* (0.2039)*	0.2041* (0.1354)*	0.2696* (0.2301)*	0.2351* (0.1281)*	0.2428* (0.2011)*	0.2241* (0.1114)*	0.2274* (0.1093)*	0.179* (0.1453)*	0.0987* (0.1052)*	
CUZ	0.2604* (0.2076)*	0.2051* (0.2158)*	0.1712* (0.1587)*	0.236* (0.2454)*	0.2567* (0.2163)*	0.2056 (0.1986)	0.2096* (0.1559)*	0.2177* (0.1637)*	0.1842* (0.2157)*	0.2416* (0.2214)*	0.2585* (0.1838)*

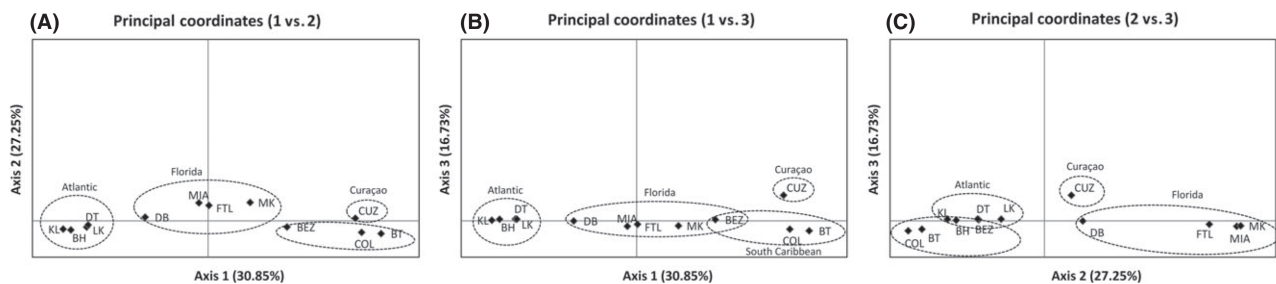

Fig. 2 A principal coordinates analyses (PCoA) using genetic distances from a population-level pairwise F_{ST} matrix. A (biplot axis 1 vs axis 2); B (biplot axis 1 vs axis 3); C (biplot axis 2 vs axis 3). BH: Bahamas (Various reefs); FTL: Florida (Fort Lauderdale); DB: Florida (Dania Beach); MIA: Florida (Miami); KL: Florida (Key Largo); MK: Florida (Middle Keys); LK: Florida (Looe Key); DT: Florida (Dry Tortugas); BEZ: Belize (Carrie Bow Cay); COL: Colombia (San Andrés Island); BT: Panama (Bocas del Toro); CUZ: Curaçao (Carmabi).

analysed (Fig. 2). Florida locations included in the Atlantic cluster (Key Largo, Looe Key and Dry Tortugas) corresponded to deeper (10–22 m depth) and farther sites (7.2–121 km) from the coastline, whereas locations in the Florida cluster (Fort Lauderdale, Miami and Middle Keys) corresponded to shallow sites (6–8 m depth) closer to the coastline (0.5–3.4 km; see Table 1).

Individual-based PCoA analyses at Greater Caribbean level also separated the three main groups observed at a population level: (i) Atlantic, (ii) Florida, and (iii) South Caribbean. This division was mainly explained by axis 1 in 11.8% and axis 2 in 9.17%. However, some Belize individuals were found in between the South Caribbean and Florida–Bahamas clusters, and all Curaçao individuals in between the South Caribbean and Florida clusters. Some individuals from the Florida cluster (Dania Beach, Fort Lauderdale, Miami and Middle Keys) were mixed with the Atlantic cluster (Bahamas, Key Largo, Looe Key, Dry Tortugas). However, only some individuals from the Atlantic cluster (from Looe

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Key) were also found in the Florida cluster (Fig. 3A–C). This was also supported when analyses were performed only including individuals from Florida and Bahamas (Fig. 3D–F) in which axis 1 explained 18.2% of the variation found in the distance matrix, and axes 2 and 3 explained 4.3% and 3.5%, respectively. At the South Caribbean level, axis 1 separated Colombia and Panama populations from Belize and Curaçao at 11.3%. Axes 2 and 3 which explained 6.5% and 5.7% of the variation did not reveal any substructure between the fourth populations (Belize, Colombia, Curaçao and Panama) (see Fig. 3G–I).

Bayesian algorithm – individual level. The Bayesian algorithm in STRUCTURE also recovered three genetic clusters across the Greater Caribbean level when using ten and six loci and both models: (i) Atlantic (Bahamas, Dry Tortugas, Key Largo and Looe Key), (ii) Florida (Fort Lauderdale, Miami and Middle Keys) and (iii) South Caribbean (Belize, Panama, Colombia and Curaçao) (Figs 4 and S1, Supporting information). Dania Beach shared both individuals belonging to the Atlantic and Florida clusters (Fig. 4A). When both models (admixture, and admixture + *locprior*) were run only with six loci, Curaçao individuals were included within the Atlantic cluster, instead of the South Caribbean (Fig. S1, Supporting information). When the analyses were performed only for Florida and Bahamas locations, both models, for both ten and six loci, showed the same two genetic clusters already found at the Greater Caribbean level: (i) Atlantic (Bahamas, Dry Tortugas, Key Largo and Looe Key) and (ii) Florida (Fort Lauderdale, Miami and Middle Keys) and Dania Beach having almost the same amount of individuals belonging to the Atlantic and to the Florida clusters (Fig. 4B). Interesting, as in the PCoA analyses, the Atlantic cluster corresponded to deeper and farter locations and the Florida cluster corresponded to shallow and closer to the coast locations

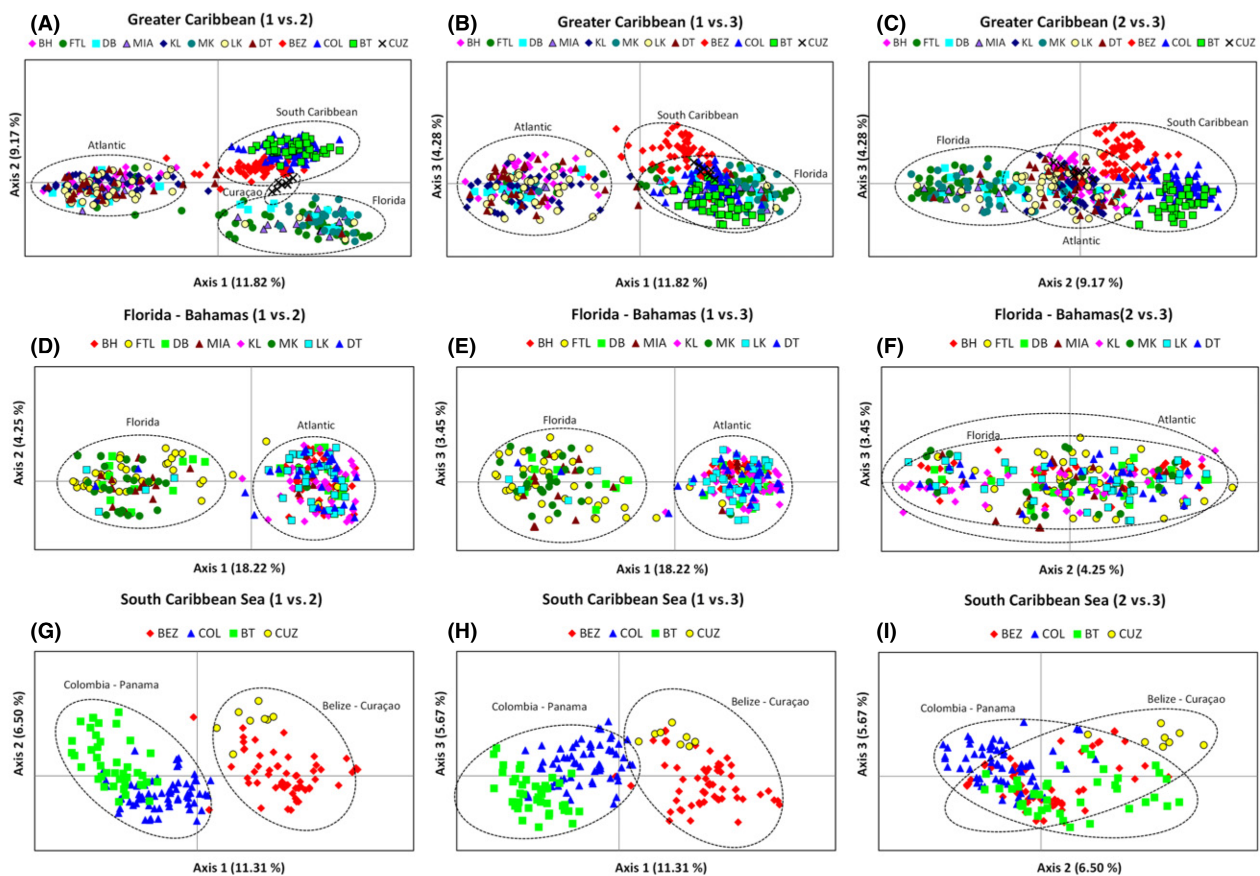

Fig. 3 Results of the Principal Coordinates Analysis (PcoA) using genetic distances between individuals and a geographic distance matrix in GENALEX 6.501. The three-axis comparisons are showed per analysis level, and the percentage of variation explained by each axis is in parentheses. Greater Caribbean Sea level (A,B,C); Florida and Bahamas (D,E,F); South Caribbean Sea (G,H,I). BH: Bahamas (Various reefs); FTL: Florida (Fort Lauderdale); DB: Florida (Dania Beach); MIA: Florida (Miami); KL: Florida (Key Largo); MK: Florida (Middle Keys); LK: Florida (Looe Key); DT: Florida (Dry Tortugas); BEZ: Belize (Carrie Bow Cay); COL: Colombia (San Andrés Island); BT: Panama (Bocas del Toro); CUZ: Curaçao (Carmabi).

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(see Table 1). At the South Caribbean level, the admixture model with both 10 and 6 loci differentiate four genetic clusters each of them corresponding to the sampling locations (Belize, Panama, Colombia and Curaçao; Fig. 4C). However, the admixture model + *locprior* with both 10 and 6 loci found only two genetic clusters, one including individuals from Belize and Curaçao and the second one including individuals from Colombia and Panama (Fig. 4C).

Iterative reallocation method – individual level. The iterative reallocation analyses by FLOCK were also in agreement with both Bayesian and multivariate analyses in finding three main genetic clusters at the Greater Caribbean level: (i) Atlantic (Bahamas, Dry Tortugas, Key Largo and Looe Key), (ii) Florida (Fort Lauderdale, Miami and Middle Keys) and (iii) South Caribbean (Belize, Panama, Colombia and Curaçao) (Figs 5A and 6). Dania Beach was a location that contained almost the same amount of individuals assigned to either the Atlantic and Florida clusters (Figs 5A and 6). When analyses were performed including only individuals from Florida and Bahamas, the same two clusters for this area were found (Atlantic and Florida) (Figs 5B

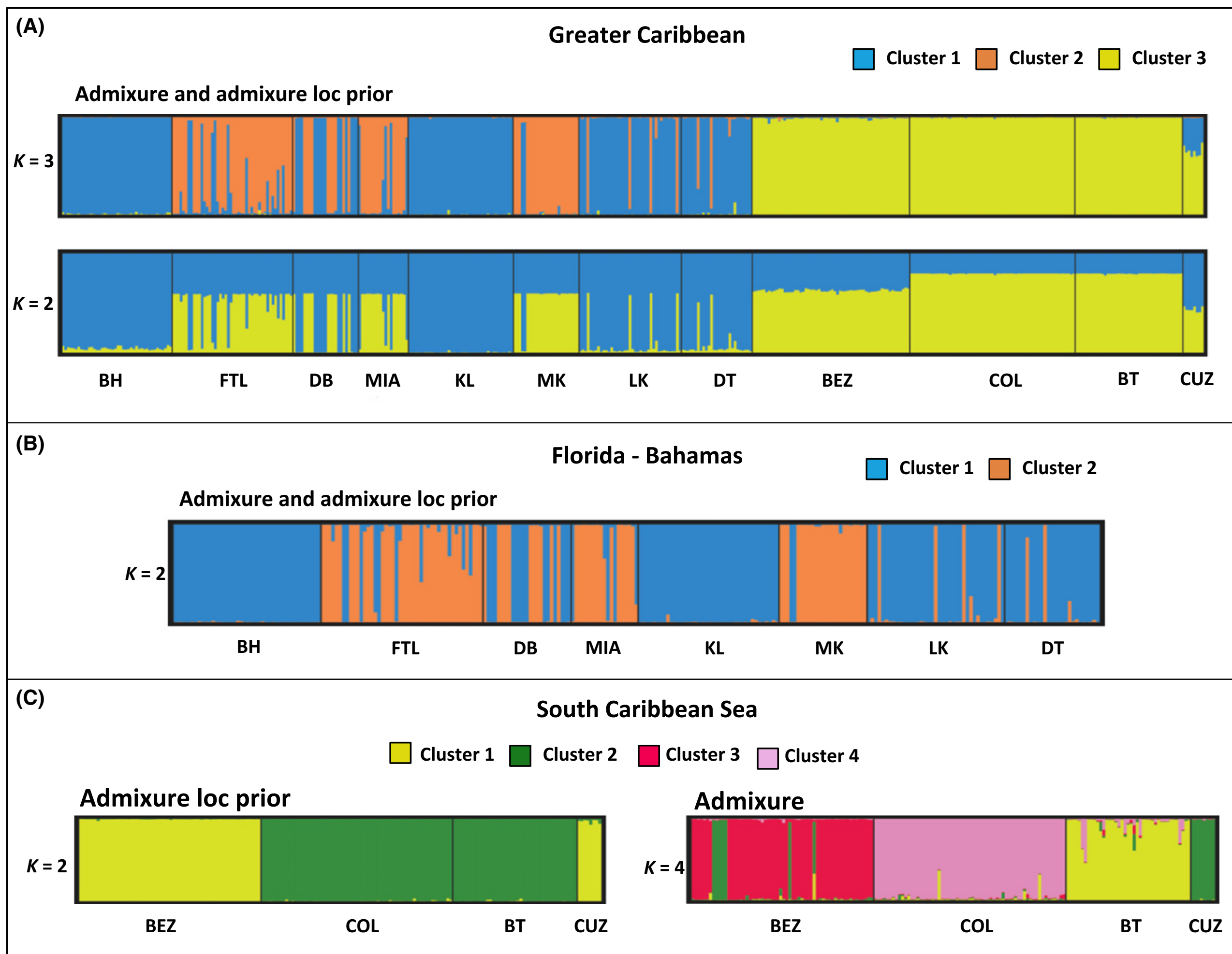

Fig. 4 Assignment of individuals to genetically homologous groups (K) as inferred by Bayesian analyses using admixture and admixture + *locprior* models in STRUCTURE, run with 10 loci for 200 000 burn-in iterations, in ten replicate sets, followed by 200 000 Markov chain Monte Carlo (MCMC) repetitions. $K = 3$ at the Greater Caribbean is the supported result by STRUCTURE, but $K = 2$ is presented below to visualize which of the clusters is the most differentiated. Each individual is represented by a vertical bar partitioned into K-coloured segments that represents its estimated membership fraction in each of the inferred groups. (A) Greater Caribbean (all locations); (B) Florida and Bahamas locations; (C) South Caribbean Sea locations. BH: Bahamas (Various reefs); FTL: Florida (Fort Lauderdale); DB: Florida (Dania Beach); MIA: Florida (Miami); KL: Florida (Key Largo); MK: Florida (Middle Keys); LK: Florida (Looe Key); DT: Florida (Dry Tortugas); BEZ: Belize (Carrie Bow Cay); COL: Colombia (San Andrés Island); BT: Panama (Bocas del Toro); CUZ: Curaçao (Carmabi).

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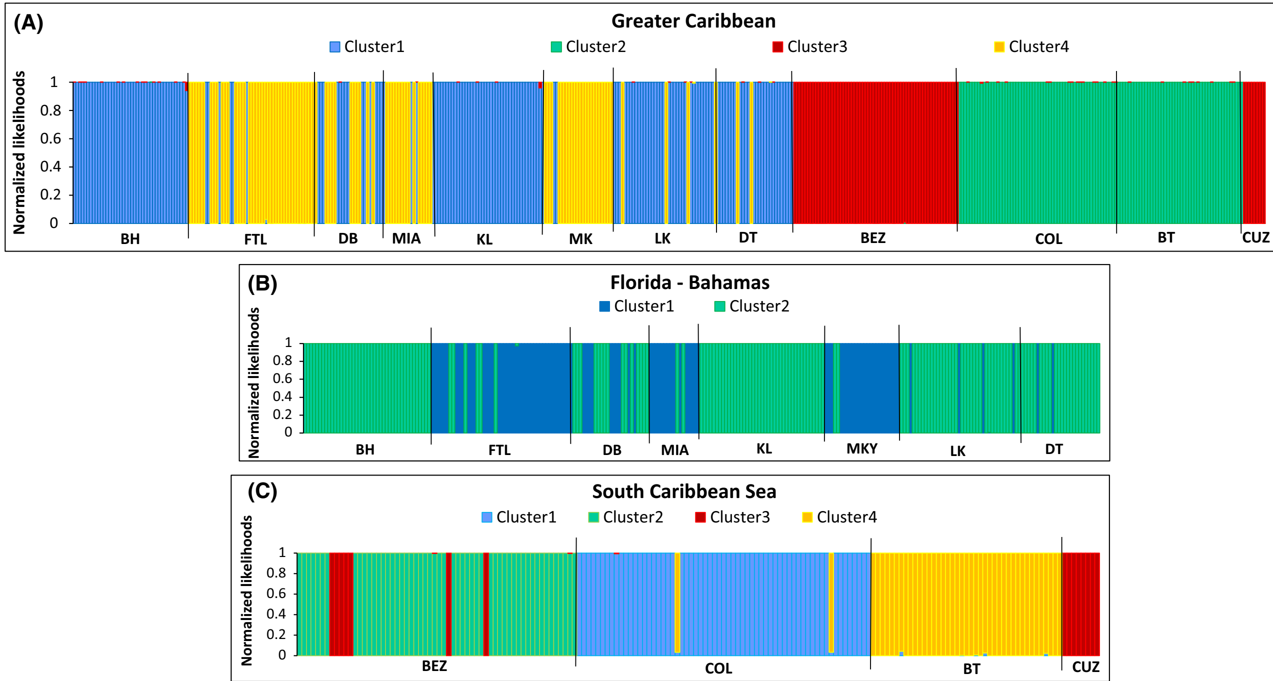

Fig. 5 Assignment of individuals based on the normalized likelihood using the iterative reallocation method of FLOCK with 10 loci. Partition of the sampled specimens was done in a random mode, with 20 iterations and 50 runs for each k assessed. (A) Greater Caribbean k = 4, (B) Florida-Bahamas k = 2, (C) South Caribbean Sea k = 4. Each line represents each individual. Geographic locations are marked in the x-axis. BH: Bahamas (Various reefs); FTL: Florida (Fort Lauderdale); DB: Florida (Dania Beach); MIA: Florida (Miami); KL: Florida (Key Largo); MK: Florida (Middle Keys); LK: Florida (Looe Key); DT: Florida (Dry Tortugas); BEZ: Belize (Carrie Bow Cay); COL: Colombia (San Andrés Island); BT: Panama (Bocas del Toro); CUZ: Curaçao (Carmabi).

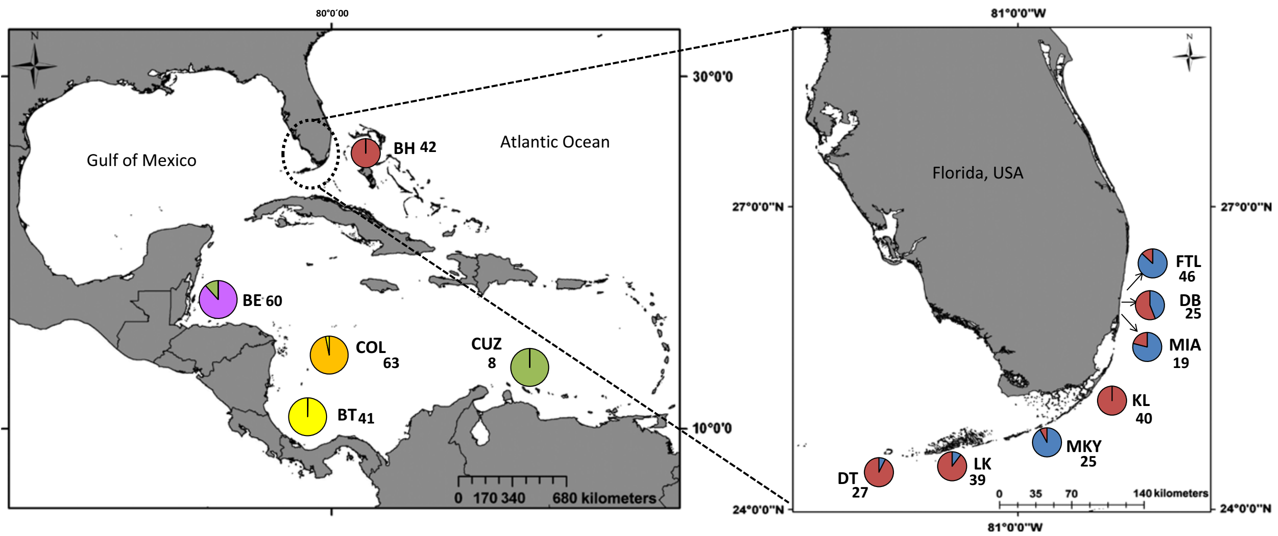

Fig. 6 Average normalized likelihood per location obtained using the iterative reallocation method in FLOCK with 10 loci. Partition of the sampled specimens was done in a random mode, with 20 iterations and 50 runs for each k assessed. Greater Caribbean k = 4, Florida–Bahamas k = 2, South Caribbean Sea k = 4. Each colour in the graph represents a different cluster, red: Atlantic cluster, blue: Florida cluster, purple: Belize cluster, orange: Colombia cluster, yellow: Panama cluster and green: Curaçao cluster. Numbers of individuals analysed on the side of each location. BH: Bahamas (Various reefs); FTL: Florida (Fort Lauderdale); DB: Florida (Dania Beach); MIA: Florida (Miami); KL: Florida (Key Largo); MK: Florida (Middle Keys); LK: Florida (Looe Key); DT: Florida (Dry Tortugas); BEZ: Belize (Carrie Bow Cay); COL: Colombia (San Andrés Island); BT: Panama (Bocas del Toro); CUZ: Curaçao (Carmabi).

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and 6). However, when only the South Caribbean locations were analysed, four different clusters were found, with each representing a specific geographical location: Belize, Colombia, Curaçao and Panama (Figs 5C and 6). The plateau records to define the number of k are included as Table S6 (Supporting information).

Dispersal patterns

Genetic isolation by geographic distance – population level. At the Greater Caribbean level, the correlation was positive (R^2 = 0.4121) and significant for genetic isolation due to geographic distance ($P \leq 0.002$). The greatest isolation was found between the Atlantic (Florida–Bahamas) and South Caribbean locations (Fig. 7A). However, subsequent tests within the Atlantic (R^2 = 0.0091; $P \leq 0.392$) and within South Caribbean locations (R^2 = 0.5785; $P \leq 0.124$) did not show significant evidence of isolation by distance (Fig. 7B, C).

Detection of first-generation migrants – individual level. From the 348 individuals included in the assignment analysis, 15.2% and 16.4% of the individuals (for 10 and 6 loci, respectively, $\alpha = 0.01$) were first-generation migrants to a new location. When α was set at 0.05, a slight increment was recorded, 17% and 18.1% (10 loci and 6 loci, respectively).

Both 10 and 6 loci ($\alpha = 0.01$) detected first migrants in all Florida locations and in the Bahamas (Table 3). But only 6 loci analysis found first migrants among Panama (α = 0.01, 0.05), Belize and Colombia ($\alpha = 0.05$) (see Table 3). Dispersal was in the same proportion either direction for both 10 loci (48% to the south and 52% to the north) and 6 loci (52% to the south and 48% to the north) at $\alpha = 0.01$, (similar percentages for $\alpha = 0.05$, Table 3). Dispersal from Florida to the Bahamas occurred specifically from three locations, Dania Beach, Key Largo and Dry Tortugas, whereas dispersal from the Bahamas to Florida only was recorded towards Key Largo (Table 3). Among the Florida reef tract, most of the dispersal occurred from four locations, Dania Beach, Key Largo, Looe Key and Middle Keys, and most of the 'migrants' were found among Key Largo, Looe Key and Dry Tortugas, whereas dispersal from Dania Beach occurred almost in the same amount towards Fort Lauderdale, Dry Tortugas, Key Largo, Looe Key and Miami. Most of migrants from Middle Keys were found in Fort Lauderdale and Miami (Table 3).

Dispersal between the South Caribbean Sea locations and the Atlantic (Florida–Bahamas) occurred (three migrants) mostly towards the north (Table 3) and from the Caribbean Sea (Curaçao, Panama and Belize) to the Bahamas. Only one individual originated in Florida (Looe Key) was found as a migrant into the South Caribbean (Colombia).

Discussion

Summary statistics

We developed 10 new microsatellite markers for the excavating sponge *Cliona delitrix*. These markers showed high levels of polymorphism allowing the determination of distinct population subdivisions across the Greater Caribbean sampling area. Although four of the microsatellite loci were in linkage disequilibrium (LD), this was not evident across all populations (see Table S3, Supporting information). Linked loci can affect population differentiation by artificially inflating levels of differentiation. For example, if two rare alleles are inherited more than expected by chance in population A, then that population may appear more differentiated due to individuals inheriting rare alleles. To be conservative with our estimates of differentiation, we ran analyses that assumed linkage equilibrium as a priori information with and without the linked loci. LD has been previously found in microsatellite markers of marine sponges (Blanquer & Uriz 2010; Richards 2010; Guardiola *et al*. 2012), and even with this condition, they have been useful to differentiate populations at temporal and spatial scales (Blanquer & Uriz 2010; Guardiola *et al*. 2012). Also, loci under LD may not

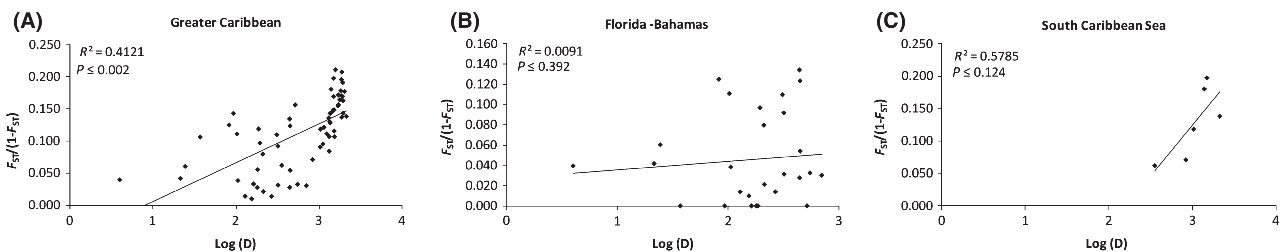

Fig. 7 Genetic isolation by distance for *Cliona delitrix* individuals inferred from multilocus estimates of genetic differentiation $F_{ST}/(1-F_{ST})$ and the logarithm of the geographic distance (D) using a Mantel test. Ten 1oci were used for this test. (A) Greater Caribbean; (B) Florida and Bahamas; and (C) the South Caribbean Sea.

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Table 3 Number of first-generation migrants with 10 loci (top table) and 6 loci (bottom table). First column α = 0.01 and second column α = 0.05. BH: Bahamas (Various reefs); FTL: Florida (Fort Lauderdale); DB: Florida (Dania Beach); MIA: Florida (Miami); KL: Florida (Key Largo); MK: Florida (Middle Keys); LK: Florida (Looe Key); DT: Florida (Dry Tortugas); BEZ: Belize (Carrie Bow Cay); COL: Colombia (San Andrés Island); BT: Panama (Bocas del Toro); CUZ: Curaçao (Carmabi). (—): no-migrants

	Populations of origin																									
	BH		FTL		DB		MIA		KL		MK		LK		DT		BEZ		COL		BT		CUZ		Total	
10 loci																										
Found in	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05	0.01	0.05
BH	—	—	—	—	1	1	—	—	3	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	4
FTL	—	—	—	—	1	1	—	1	1	1	4	4	2	2	—	—	—	—	—	—	—	—	—	—	8	9
DB	—	—	2	2	—	—	—	—	5	5	2	2	1	1	—	—	—	—	—	—	—	—	—	—	10	10
MIA	—	—	—	—	2	2	—	—	—	—	3	3	—	—	—	—	—	—	—	—	—	—	—	—	5	5
KL	1	2	—	—	1	2	—	—	—	—	—	—	1	1	3	3	—	—	—	—	—	—	—	—	6	8
MK	—	—	1	1	—	—	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	3
LK	—	—	—	1	2	3	—	—	6	7	2	2	—	—	—	—	—	—	—	—	—	—	—	—	10	13
DT	—	—	—	—	1	1	—	—	2	3	—	—	3	3	—	—	—	—	—	—	—	—	—	—	6	7
BEZ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
COL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
BT	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
CUZ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	1	2	3	4	8	10	2	3	17	19	11	11	7	7	3	3	—	—	—	—	—	—	—	—	52	59
6 loci																										
BH	—	—	—	—	—	—	—	—	3	4	—	—	—	—	—	1	1	1	—	—	1	1	1	1	6	8
FTL	—	—	—	—	1	1	—	—	1	1	1	2	3	3	—	—	—	—	—	—	—	—	—	—	6	7
DB	—	—	3	3	—	—	—	—	1	1	—	—	3	3	1	1	—	—	—	—	—	—	—	—	8	8
MIA	—	—	—	—	2	2	—	—	—	—	3	3	—	—	—	—	—	—	—	—	—	—	—	—	5	5
KL	1	2	—	—	1	1	—	—	—	—	—	—	3	4	3	3	—	—	—	—	—	—	—	—	8	10
MK	—	—	2	2	—	—	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	4
LK	—	—	1	1	3	3	—	—	4	4	2	2	—	—	—	1	—	—	—	—	—	—	—	—	10	11
DT	—	—	—	—	1	1	—	—	3	3	—	—	3	3	—	—	—	—	—	—	—	—	—	—	7	7
BEZ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	1	1
COL	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	1
BT	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	1	1
CUZ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	1	2	6	6	8	8	2	2	12	13	6	7	12	14	4	6	1	1	1	1	2	2	1	1	56	63

Total dispersal at α = 0.01: 48% to the south and 52% to the north; at α = 0.05: 53% to the south and 47% to the north (10 loci).
Total dispersal at α = 0.01: 52% to the south and 48% to the north; at α = 0.05: 49% to the south and 51% to the north (6 loci).

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necessarily maintain in the LD condition over time (Guardiola *et al.* 2012). In addition, linkage at a global level does not necessarily reflect LD across all populations as we found for *C. delitrix* and Blanquer & Uriz (2010) for the sponge *Scopalina lophyropoda*.

Mean expected heterozygosities for all loci in *Cliona delitrix* (H_E = 0.592–0.805) were within the range of other marine sponges: *Crambe crambe* (H_E = 0.422–0.748) (Duran *et al.* 2004), *Paraleucilla magna* (0.609–0.698) (Guardiola *et al.* 2012), *Spongia officinalis* (H_E = 0.73–0.90) (Dailianis *et al.* 2011) and *Xestospongia muta* (0.640–0.719) (Richards 2010). Also, as commonly observed in sponges, *C. delitrix* showed departure from HWE, significant heterozygote deficiency for all 10 loci and within all locations. This deficiency was revealed in high positive F_{IS} values – suggesting nonrandom mating between individuals – which could have resulted for technical reasons such as the presence of nonamplifying alleles (i.e. null alleles). Null alleles can commonly occur in invertebrates due to mutations in the flanking regions of microsatellites (Callen *et al.* 1993; McGoldrick *et al.* 2000; Reece *et al.* 2004; Brownlow *et al.* 2008) or to biological reasons such as inbreeding, selfing and Wahlund effects (Freeland 2005; Allendorf & Gordon 2007). When exploring the effects of null alleles, we found that the presence of average null alleles for each locus was lower (6.8% overall) than for the sponge *Xestospongia muta* (13.1%) (Richards 2010) or other invertebrates as the Barnacle *Pollicipes elegans* (12%) (Plough & Marko 2014). But, the higher F_{IS} values and heterozygosities deficiencies in *C. delitrix* could be due to biological reasons. For example, inbreeding cannot be completely ruled out. If we consider that *C. delitrix* larvae behave similarly to other *Cliona* larvae (crawling with low dispersal capabilities), then philopatry could be generated (Warburton 1966; Mariani *et al.* 2000). This is a typical condition of sponge larvae that structure populations (Debiasse *et al.* 2010; Dailianis *et al.* 2011; Guardiola *et al.* 2012). In addition, *C. delitrix* populations have a small percentage of hermaphroditic individuals (Chaves-Fonnegra 2014) and inbreeding could be the result of self-fertilization. Larvae in *Cliona* sponges can fuse before starting to swim (Warburton 1958) and may form chimeras, but heterozygosity deficiencies reflect that this may not be the case for *C. delitrix*. Sponge chimeric individuals, despite their philopatric larvae and small patchy populations, exhibit heterozygote excess and high genetic diversity (Blanquer & Uriz 2011).

In addition, heterozygote deficiency in *Cliona delitrix* can be explained by a temporary Wahlund effect, as a consequence of different breeding subunits within each sampled location, as seen in the Dania Beach location (Florida). This may be possible as *C. delitrix* has an asynchronous reproduction with multiple peaks of gametes released, in which not all individuals in each location engage in reproduction (Chaves-Fonnegra 2014). Therefore, each location could have several breeding subunits of individuals reproducing at different times over years. This characteristic has been suggested previously for *Crambe crambe*, a sponge that may present a reproductive lag between subpopulations, allowing the formation of different breeding units (Uriz *et al.* 1998; Duran *et al.* 2004).

The South Caribbean locations and Bahamas had a greater number and higher average frequency of private alleles than any of the Florida locations. This can indicate a recent expansion of the species to Florida reefs, which are marginal to the distribution range of *C. delitrix* (see map in van Soest *et al.* 2013). This has been suggested for *Crambe crambe* in the Mediterranean (Duran *et al.* 2004). Private alleles can also be estimators of gene flow (Slatkin 1985). The low frequency of private alleles in Florida locations may be the result of high gene flow between these locations, whereas isolated populations (e.g. South Caribbean Sea) have accumulated private alleles over time.

Greater Caribbean

Fisher's exact test (F_{ST} values) supported Bahamas, Belize, Colombia and Panama as independent clusters. In Florida, some locations were genetically similar. Curaçao was connected to Florida (Middle Keys location). Bayesian, multivariate and iterative reallocation methods supported differentiation between Atlantic locations (Florida and Bahamas) and the South Caribbean locations (Belize, Colombia, Curaçao and Panama), and also within Florida. Although the Bayesian method required loci to be in HWE and linkage equilibrium as a priori information, this analysis was not affected by the presence of linked loci, as it showed similar results with 6 and 10 loci, and to the multivariate and iterative methods that do not require HWE or LD as a priori assumptions.

Isolation by geographic distance was found between the Atlantic and South Caribbean locations. However, first migrant analyses (6 loci) suggest limited dispersal may be possible between these geographic areas (over 1100 km). Previous genetic studies through mtDNA sequences and microsatellite allele frequencies showed that dispersal of *Xestospongia muta* between Honduras/Bahamas locations and Florida had occurred for thousands of generations (Lopez-Legentil & Pawlik 2009; Richards 2010). As for *C. delitrix*, contemporary dispersal for *X. muta* between these geographic locations (over 1000 km apart) is unlikely (Richards 2010). The Caribbean and Loop currents are the main surface circulation

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able to disperse pelagic larvae of invertebrates and connect populations between the Caribbean Sea and Florida (Mitton *et al.* 1989; Silberman *et al.* 1994; see Fig. 1). However, for sponges with low dispersal larvae (Uriz *et al.* 1998; Mariani *et al.* 2000; Maldonado 2006), the speed of the Caribbean Current (31 cm/s between Aves Ridge, west of the Lesser Antilles and the Florida Straits) (Fratantoni 2001) vs. the time of the larvae in the water column is not enough to maintain a constant genetic exchange between the Caribbean Sea and Florida–Bahamas area (Fig. 1). If *C. delitrix* larvae behave similarly to *C. celata* and *C. viridis* (retention in the water column between 24 h and 10 days), its dispersal from Curaçao to Florida could be between 27 and 269 km. This suggests that direct interchange between Curaçao and Florida locations through the Caribbean Current (distance over~ 2023 km) is unlikely, as is gene flow between Belize and Florida (over~ 1000 km).

Atlantic (Florida–Bahamas)

F_{ST} values and Fisher's exact test supported a genetic cluster in Florida, conformed by Dry Tortugas, Key Largo and Looe Key. However, genetic similarity was found between Key Largo and Dania Beach, the Middle keys and Curaçao, Miami and Fort Lauderdale, and Fort Lauderdale and Middle Keys. Bahamas was found as an independent cluster (for both 10 and 6 loci analyses). In contrast, Bayesian, multivariate and iterative reallocation methods supported two genetic clusters in Florida, with one of them including the Bahamas: (i) Atlantic (Bahamas, Dry Tortugas, Key Largo, Looe Key) and (ii) Florida (Fort Lauderdale, Miami and Middle Keys). Dania Beach was admixed with several individuals from both clusters. In the Atlantic cluster, the Florida locations (Dry Tortugas, Key Largo and Looe Key) were all deeper sites (10–22 m depth) and farther from the coastline (7.2–121 km). Whereas locations in the Florida cluster (Fort Lauderdale, Miami and Middle Keys) were shallow sites (6–8 m depth) closer to the coastline (0.5–3.4 km; Table 1). Thus, depth appears to be one of the factors differentiating populations of *C. delitrix* in Florida. These results agree with a recent study on corals in Florida (Serrano *et al.* 2014), in which *Montastraea cavernosa* is differentiated in two genetic clusters, one shallow (≤10 m) and one deeper (≥15–25 m), whereas in *Porites asteroides,* the depths at which the two genetic clusters differentiate varied regionally: in the Upper and Lower Keys, at ≥15 m, while in the Dry Tortugas, at ≥25 m (Serrano 2013; Serrano *et al.* 2014). Thus, our findings with *C. delitrix* support depth as an important factor structuring invertebrate populations in the Florida reef tract. This is also the first time in which depth is found as a potential factor affecting the structure of sponge populations in coral reefs. For example, individuals occurring in deeper locations in Florida cluster with individuals from Bahamas, suggesting connectivity between Florida and Bahamas may occur through exchanges of deep-water currents. In Dania Beach (at 9 m depth; Table 1), our analyses showed a clear overlap of the two genetic clusters. Thus, we posit that besides depth, a Wahlund effect may be occurring for *C. delitrix* populations in the Florida reef tract. In this geographic area, it is possible that each genetic cluster (shallow/deep) represents different breeding subunits that reproduce at different times due to the asynchronous multispawning reproduction in *C. delitrix* (Chaves-Fonnegra 2014). The overlap between these breeding subunits tends to be greater in the northern locations (Dania Beach and Fort Lauderdale), where the continental shelf is narrow (<5 km) (Lee 1975) and both deep and shallow reefs are closer to each other and to the coastline than in the Florida Keys (~ 7–10 km) (Lee & Williams 1999).

Isolation by geographic distance was not significant within the Florida reef tract, supporting that in this region, other factors – possibly depth and reproductive strategy – are determining the structure of populations. The first-generation migrants' analysis suggests that dispersal and connectivity occur along the~ 315 km of the Florida reefs tract sampled. *Cliona delitrix* showed higher dispersal than *Xestospongia muta* (Richards 2010) and *Callyspongia vaginalis* (Debiasse *et al.* 2010) along Florida reefs. Dispersal occurred between locations spanning a minimum distance of ~ 3 km (Dania Beach to Fort Lauderdale) and up to ~ 315 km (Dania Beach to Dry Tortugas). These results support previous studies suggesting higher connectivity of sponges and corals along the Florida coast (Baums *et al.* 2010; Richards 2010; Andras *et al.* 2013) where the relatively strong Florida Current (200 cm/s) and Tortugas Gyre (40 cm/s) (Lee *et al.* 1994; Lee & Williams 1999) could be responsible for transporting eggs or larvae of *C. delitrix* along the Florida reef tract (see Fig. 1), as previously suggested for other invertebrates and fish (Lee *et al.* 1994; Criales & Lee 1995; Lee & Williams 1999; Richards 2010; Serrano *et al.* 2014). The strong connectivity and dispersal found for *C. delitrix* between Dry Tortugas, Looe Key and Key Largo may be facilitated by the Tortugas Gyre, which moves from Dry Tortugas into the vicinity of Looe reef (Lee *et al.* 1994), and simultaneously with the Florida Current, which takes a northward direction from the Middle Keys to the Upper Keys (Lee & Williams 1999). Interestingly, the Tortugas Gyre influences on water motions in the Keys extend from May to November (see map and descriptions in Lee *et al.* 1992, 1994; Lee & Williams 1999), the same

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period in which the reproductive cycle of *C. delitrix* occurs in Florida (Chaves-Fonnegra 2014).

Our study also supports connectivity between the deeper (and further locations from the coast) in the Florida reef tract and the Bahamas. One previous study with the sea fan *Gorgonia ventalina* also supports connectivity across the Florida Current (over ~ 88 km distance) (Andras *et al.* 2013). These results contrast with previous studies in the area, which found the Florida Current as a barrier that impedes larvae exchange in sponges and corals (Vollmer & Palumbi 2007; Lopez-Legentil & Pawlik 2009; Baums *et al.* 2010; Richards 2010). However, the northeasterly direction of the Florida Current in Key Largo could facilitate the interchange of larvae outside Florida and specifically to the Bahamas (Lee & Williams 1999). Due to the change and intensification of the Florida Current in the Upper Keys (Lee & Williams 1999), we consider this area (specifically Key Largo) is exposed to more larval exchange from populations outside the Florida reef tract area, as previously suggested for *Xestospongia muta* (Richards 2010). Connectivity across the Florida Current is feasible, although it is probably constrained by the complexity of the ocean currents in the area, and the period of time in which reproduction and dispersal of the populations occur. Depth also could be the main factor segregating populations in this region, as found at a greater scale (Caribbean Sea) for the candelabrum coral *Eunicea flexuosa* (Prada & Hellberg 2013). Future studies between Florida and Bahamas could address this hypothesis.

Cliona delitrix dispersal was in equal proportions in both directions (south/north) of the Florida reef tract. Connectivity in both directions and between the Upper Keys (Key Largo) and Northern reef tract locations (Miami, Dania Beach and Fort Lauderdale) could be possible through the Florida Current, whereas dispersal from the north locations to the south could be attributed to the Coastal Countercurrent, eddies and gyres that form closer to the coast (Lee 1975; Lee & Mayer 1977; Lee & Williams 1999). Also, it is feasible that the deeper and farther locations in the Atlantic cluster are influenced by the Florida Current, which flows separated from the coast (Lee & Williams 1999), whereas the shallow and closer to the coast locations (Florida cluster) are connected by the Coastal Countercurrent that circulates near to the coast (Lee & Mayer 1977).

South Caribbean Sea

We found two to four genetic clusters in the South Caribbean Sea. The PCoA method separated two genetic populations: (i) Colombia, Panama and Belize as one population and (ii) Curaçao. In contrast, the Bayesian and iterative analyses together supported four South Caribbean geographic locations as genetic populations: (i) Belize, (ii) Colombia, (iii) Curaçao and (iv) Panama. The F_{ST} values and Fisher's exact test supported the four genetic clusters, although Curaçao was linked to the Middle Keys location in Florida. Therefore, we consider that South Caribbean locations form independent populations, but Colombia, Panama and Belize (southwest Caribbean) showed more connectivity and some admixture between them. These results are consistent with previous studies of the Sea fan coral *Gorgonia ventalina*, which also showed apparent admixture among Panama and the Mesoamerican Barrier Reef (=localities among Belize and Mexico coast) (Andras *et al.* 2013). It is possible that the Belize–Curaçao cluster suggested by the *loc prior* Bayesian analysis and the Middle Keys–Curaçao cluster suggested by the F_{ST} Fisher's exact tests are an artefact due to insufficient sampling from Curaçao (n = 8), as both PCoA and iterative methods designated this location an independent genetic population.

Although the four sampled locations in the South Caribbean Sea constitute different genetic populations, isolation by distance was not significant between them (along ~ 2100 km). Thus, as in the Atlantic (Florida–Bahamas locations), it is possible that other factor (i.e. depth) is structuring populations (see Prada & Hellberg 2013). Gene flow in the South Caribbean area is present and first-generation migrants between Colombia and Panama, and Panama and Belize were detected in the analysis with six unlinked loci. Connectivity in this area is possible as the Caribbean Current velocities along the coasts of Venezuela and the Netherland Antilles can reach 70 cm/s (Fratantoni 2001), and an intense recirculation gyre (60 cm/s) drives strong currents in the southwest corner of the Colombian Basin (80° W, 12° N) (Fratantoni 2001). If we consider the speed of the gyre and estimate the time of larvae retention between 24 h and 10 days (Warburton 1966; Mariani *et al.* 2000), dispersal distances could reach 51–518 km; therefore, it is likely that larvae of *C.delitrix* are being interchanged between Bocas del Toro, Panama and San Andres Island, Colombia (~ 345 km linear distance, and ~ 542 km semicircle perimeter). Although less feasible, larvae may be transported by eddies between Panama and Colombia to the Mesoamerican Barrier Reef.

Dispersal of C. delitrix on coral reefs

Using allozymes, Zilberberg et al. (2006) suggested dispersal ranges for *Cliona delitrix* larger than 10–100 m, and our results, employing microsatellites, support this hypothesis. Dispersal of *C. delitrix* may reach as far as ~350 km (Florida reef track and between Colombia and Panama), and could be favored especially where strong currents are able to transport sponge eggs or larvae over

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longer distances. This species is a strong competitor of corals and can take advantage of recent coral mortality (Chaves-Fonnegra 2014). Additionally, *C. delitrix* as other species of excavating sponges, may survive coral bleaching events and resist more acidic environments (Wisshak *et al.* 2012; Carballo *et al.* 2013; Stubler *et al.* 2014), becoming an important community component of reef habitats. Results presented here can be used as a baseline to monitor future coral reef trends under climate change.

Acknowledgements

These results were presented by A. C-F as part of her PhD dissertation in NOVA Southeastern University, Oceanographic Center, FL, USA. This work was supported by the UNESCO-L'Oréal Fellowship for Young Women in Science, the PhD scholarship programme from Colombian Science and Technology Department (COLCIENCIAS), Billfish Tournament Scholarship and the Broward Women Association Scholarship. Fieldwork was possible in the Bahamas and Florida thanks to Joe Pawlik, members of the 2011 Chemical Ecology of Marine Organisms Cruise, Sven Zea, Malcom Hill, Rob Ruzicka, Charles Messing, John Hocevar and Dave Gilliam; in Belize to Elizabeth L. McLean, Klaus Rützler and the Carry Bow Smithsonian Marine Station; in Colombia to Sven Zea, Banda Dive Shop and the Universidad Nacional de Colombia; in Panama to Bob Thacker, Cristina Díaz, Ewelina Rubin, Tse-Lyn Loh, Jan Vicente and the Smithsonian Station at Bocas del Toro; and in Curaçao to Benjamin Müller and the CARMABI Research Station. Many thanks to all members of the NSU Oceanographic Center Laboratories: Marine Microbiology and Genetics, Conservation Biology and Genetics, and Coral Reef Restoration and Monitoring. Special thanks to Pierre Duchesne for guidance with running and interpreting the results from FLOCK program, and Andrea Bernard for guidance with PCoA analyses and Structure program.

Collecting permits for each location: Bahamas (*R/V F.G. Walton Smith*, CY 2011 'Blanket' Research Permit from the Government of the Bahamas), Florida (Fishing Permit at reef sites outside any marine protected area), except for Dry Tortugas (Greenpeace 2010 Expedition, permit DRTO-2010-SCI-0014), Belize (Belize Fisheries Department GEN/FIS/15/04/2010-53-Vol.VIII), Colombia (Universidad Nacional de Colombia's Institutional collecting permit, formalized and renewed by Resolution 0255 of March 14, 2014 of ANLA -National Authority for Environmental Licenses-), Panama (Autoridad de los Recursos Acuáticos de Panamá Resolución DGOMI-PEFC N°30 de 03 de Septiembre de 2009), and Curaçao (Annual Research Permit issued by the Curaçao Ministry of Health, Environment and Nature -GMN- to the CARMABI foundation).

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A.C-F. and J.V.L. Research concept and design; A.C-F., K.A.F., J.S. and J.V.L. Collection and/or assembly of data; A.C-F. and K.A.F. Data analysis and interpretation; A.C-F. and K.A.F. Statistical analysis; A.C-F. Writing the article; A.C-F., K.A.F. and J.V.L. Critical revision of the article and A.C-F., K.A.F., J.S. and J.V.L. Final approval of the article

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Data accessibility

Microsatellite data were deposited into Dryad. doi:10.5061/dryad.8942f.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 DRYAD file.

Fig. S1 Assignment of *Cliona delitrix* individual genotypes to genetically homologous groups (K) as inferred by Bayesian analyses using admixture and admixture + *locprior* models in STRUCTURE, with 6 unlinked loci for 200 000 burn-in iterations, in ten replicate sets, followed by 200 000 Markov chain Monte Carlo (MCMC) repetitions.

Table S1 Supporting information. Characteristics of the 10 new microsatellites loci isolated from *Cliona delitrix*.

Table S2 Cross amplification success of the 10 loci from *Cliona delitrix* on eight individuals (n = 8) of *C. laticavicola*.

Table S3 Unbiased exact tests *P*-values obtained to test for Linkage equilibrium (LE) as implemented in GENEPOP v.4.2 on the web (Raymond and Rousset 1995; Rousset 2008).

Table S4 Linkage disequilibrium correlation coefficient (r_{LD}) for all microsatellite loci pairs (using10 loci) calculated by location and globally.

Table S5 Summary of genetic variation and null allele frequencies at each locus.

Table S6 Plateau records to define the number of genetic clusters based on iterative reallocation by Paetkau multilocus maximum likelihood implemented in FLOCK 3.1. (Duchesne & Turgeon 2012).

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