Population genetic structure of the vase sponge *Ircinia campana* in the Greater Caribbean is shaped by oceanographic features and limited dispersal

Sarah M. Griffiths[1], Mark J. Butler IV[2], Donald C. Behringer[3,4], Thierry Pérez[5] & Richard F. Preziosi[1]

[1] Ecology and Environment Research Centre, Manchester Metropolitan University, Manchester, UK.

[2] Institute of Environment, Department of Biological Sciences, Florida International University, North Miami, Florida, USA.

[3] Fisheries and Aquatic Sciences, University of Florida, Gainesville, Florida, USA.

[4] Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA.

[5] Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale, Aix Marseille Université, Marseille, France.

Corresponding author: Sarah M Griffiths, John Dalton Building, Chester Street, Manchester, M1 5GD. +44 (0)161 247 3669. sarah.griffiths@mmu.ac.uk

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Abstract

Understanding population genetic structure can help us to infer dispersal patterns, predict population resilience, and design effective management strategies. For sessile species with limited dispersal this is especially pertinent because genetic diversity and connectivity are key aspects of their resilience to environmental stressors. Here, we describe the population structure of *Ircinia campana*; a common Caribbean sponge subject to mass mortalities and disease. Microsatellites were used to genotype 440 individuals from 19 sites throughout the Greater Caribbean. We found strong genetic structure across the region, and significant isolation by distance across the Lesser Antilles, highlighting the influence of limited larval dispersal. We also observed spatial genetic structure patterns congruent with oceanography. This includes evidence of connectivity between sponges in the Florida Keys and the southeast coast of the USA (>700 km away) where the oceanographic environment is dominated by the strong Florida Current. Conversely, the population in southern Belize was strongly differentiated from all other sites, consistent with the presence of dispersal-limiting oceanographic features, including the Gulf of Honduras gyre. At smaller spatial scales (<100 km), sites showed heterogeneous patterns of low-level but significant genetic differentiation (chaotic genetic patchiness), indicative of temporal variability in recruitment or local selective pressures. Genetic diversity was similar across sites, but there was evidence of a genetic bottleneck at one site in Florida where past mass

mortalities have occurred. These findings underscore the relationship between regional oceanography and weak larval dispersal in explaining population genetic patterns, and could inform conservation management of the species.

Keywords: (3-6) Porifera; genetic diversity; conservation; cryptic lineage; connectivity

Introduction

The spatial distribution of genetic diversity is influenced by both ecological and evolutionary processes, and can be used to infer a number of important characteristics of species, including dispersal, recruitment and gene flow among populations (connectivity). Understanding these processes can help in ecosystem management and conservation (Baums 2008; Almany et al. 2009). In addition, identifying changes in genetic diversity, population bottlenecks, and inbreeding is vital in the face of declining populations and environmental change, given their effects on population resilience, adaptive potential and fitness.

In Greater Caribbean marine ecosystems, sponges are abundant, diverse, and serve important ecological functions, including cycling of organic material and habitat provisioning (Diaz and Rutzler 2001; Bell 2008; Valentine and Butler 2019). Sponges form a major component of the benthos in many coral reef ecosystems, and can dominate in shallow hard bottom lagoons (Bertelsen et al. 2009). Despite their prominence and importance, relatively little is known about population structure in Caribbean sponges. Furthermore, in some localities sponges have suffered mass mortality events (Butler et al. 1995; Wulff 2006), including in the Florida Keys, where recurring die-offs of the sponge community have had

numerous negative consequences for associated communities (Butler et al. 1995, 2016; Herrnkind et al. 1997). Increasing our knowledge of the factors driving population structure in sponges is important in understanding their recruitment and dispersal patterns, and may be important for creating ecosystem-based management plans for the region.

In marine systems, population genetic structure is shaped by a dynamic interplay between life history, oceanographic features, and demographic stochasticity (Knutsen et al. 2003; Hoffman et al. 2011). As sponges are sessile for the majority of their life cycle, dispersal at the larval stage is crucial in shaping their population structure. The larval phase is generally very short in sponges, ranging from a few hours to a few days (Maldonado and Riesgo 2008), suggesting philopatry should be high (with a few known exceptions in the hoplitomella larvae; Vacelet 1999). This life history mode can result in more fragmented and isolated populations, as it limits dispersal and thus connectivity among populations (Shanks 2009). However, ocean currents can potentially increase connectivity among distant locations (Chaves-Fonnegra et al. 2015; Richards et al. 2016). Furthermore, strong wave action, predation, and discards from commercial sponge fishing produce fragments, which may disperse before reattaching to the substrate (Wulff 1991; Maldonado and Uriz 1999; Butler et al 2017). However, temporal variation in ocean circulation, as well as demographic stochasticity, can yield unpredictable and chaotic patterns of population structure (Siegel et al. 2008; Castorani et al. 2017; Drury et al. 2018).

To date, empirical population genetics studies have been conducted on only four Caribbean sponge species (*Xestospongia muta*: López-Legentil and Pawlik 2009; de Bakker et al. 2016; Richards et al. 2016; *Callyspongia vaginalis*: DeBiasse et al. 2010; *Cliona delitrix*: Chaves-Fonnegra et al. 2015; *Spheciospongia vesparium*: Griffiths et al. 2020). In general, these studies have found strongly differentiated populations throughout the region, including among sites only tens of kilometres apart in some instances. However, patterns of regional genetic structure often reflect major ocean currents and hydrology (López-Legentil and Pawlik 2009; Chaves-Fonnegra et al. 2015; Richards et al. 2016; Griffiths et al. 2020), and evidence of long distance dispersal has been found, suggesting irregular transport of larvae, gametes or sponge fragments in ocean currents over large oceanographic distances (DeBiasse et al. 2010; Chaves-Fonnegra et al. 2015). These studies reveal the complexities of sponge population genetics in the Caribbean, highlighting the need for further research on this understudied taxon, including locations that have not yet been sampled.

In this study, we explore population genetic structure in the Caribbean vase sponge *Ircinia campana* (Lamarck, 1814), a common species in coral reef and hard bottom habitats. The reproduction and larval biology of *I. campana* has not yet been described; however, other *Ircinia* species are viviparous, with no true asexual reproduction (through budding or

146 gemmules), and the vase-shaped morphology of *I. campana* is not
147 susceptible to fragmentation during storms. This species is prone to
148 disease outbreaks, and has suffered a number of mass mortality events,
149 the most extensive having occurred in the Florida Keys (Butler et al. 1995).
150 Due to both its prevalence in the region's benthic communities and its
151 apparent vulnerability, it is important to understand the genetic diversity and
152 structure of *I. campana* in the Greater Caribbean. Here, we aimed to
153 describe the population structure of *I. campana* and explore the effects of
154 mass mortalities on genetic diversity.
155
156 **Material and Methods**
157
158 **Sampling**
159 We sampled 10 - 41 *I. campana* individuals at 19 sites throughout the
160 Greater Caribbean (Table 1, Fig. 1) from depths of 1 - 25 m, resulting in a
161 total sample size of 440 individual sponges. Sponge communities, including
162 *I. campana*, at two of the sampling sites (Bamboo Key and Long Key in the
163 Florida Keys) have previously been affected by mass mortalities associated
164 with cyanobacterial blooms. We cut small fragments of tissue (~ 1.5 cm^3)
165 and preserved them in 95% ethanol soon after surfacing. The ethanol was
166 replaced after 24 h to avoid dilution with the seawater held in the sponge
167 tissue.
168
169 **Microsatellite genotyping**

We dissected samples under a stereomicroscope to remove macroinvertebrates from the sponge tissue, and extracted DNA using the DNeasy® Blood and Tissue Kit (Qiagen). We genotyped the samples for 10 microsatellite loci, as described in Griffiths et al. (2019). Briefly, we amplified the loci in two multiplex PCR reactions (Griffiths et al. 2019) with the Type-it Microsatellite PCR Kit (Qiagen), using the following thermal cycling conditions: initial denaturation of 95°C for 5 minutes, 28 cycles of 95°C for 30 seconds, 60°C (Multiplex A) or 63°C (Multiplex B) for 90 seconds, and 72°C for 30 seconds, followed by a final extension of 60°C for 30 minutes. Alleles were then sized using capillary electrophoresis on a DNA Analyzer 3730 (Thermo Fisher Scientific), and scored using Genemapper v3.7 (Thermo Fisher Scientific). All plates for PCR and genotyping contained positive and negative controls.

Quality control and summary statistics

We tested linkage disequilibrium between loci using Genepop on the Web v4.2 (Raymond and Rousset 1995), and corrected significance for multiple tests (Benjamini and Yekutieli 2001; B-Y correction) using the *p.adjust* function in R 3.4.3 (R Core Team 2017). We calculated null allele frequencies in FreeNA (Chapuis and Estoup 2007) using the Expectation Maximisation algorithm (Dempster et al. 1977), and then conducted a post hoc analysis to determine the influence of null alleles on population differentiation estimation. This analysis was carried out by calculating global F_{ST} with and without correction for null alleles using the ENA method

194 of Chapuis and Estoup (2007). Following this, the locus with the highest
195 null allele frequency was removed and F_{ST} with and without ENA correction
196 was recalculated (*sensu* Chaves-Fonnegra et al. 2015). This was repeated
197 sequentially until only one locus remained. This analysis revealed that the
198 two loci with the highest null allele frequencies, Icam34 and Icam10,
199 skewed uncorrected global F_{ST} estimates over 0.01 (Table S1).
200 Consequently, in analyses where correction for null alleles could be
201 implemented (F_{ST} and population average inbreeding coefficient (F_{IS})
202 calculations), the full 10 loci dataset was used. In all other analyses Icam34
203 and Icam10 were excluded, yielding an 8 loci dataset.
204
205 We calculated probability of deviation from Hardy-Weinberg Equilibrium
206 (HWE), observed heterozygosity (H_O) and Nei's gene diversity (H_S)
207 (expected heterozygosity) for each sampling site using GenoDive v2.0b23
208 (Meirmans and Van Tiendener 2004). We calculated the average F_{IS} for
209 each site corrected for null alleles in INEst v2.1 (Chybicki and Burczyk
210 2009), which uses a likelihood-based method to estimate F_{IS} and null allele
211 frequencies simultaneously. We used the Interacting Multiple Model (IMM)
212 with 500,000 Markov chain Monte Carlo (MCMC) cycles and 50,000 burn-in
213 cycles. We ran the model using all combinations of parameters for possible
214 null allele causes ('n': null alleles; 'b': genotyping failure; 'f': inbreeding),
215 and used Bayesian deviance information criterion (DIC) to infer which
216 parameters contributed more to the observed data.
217

Genetic diversity and bottlenecks

We assessed genetic diversity of the sites using ADZE (Szpiech et al. 2008), which uses a rarefaction method to calculate allelic richness and private allelic richness corrected for sample size variation. Gray's Reef was excluded from this analysis due to its small sample size, and because the marker Icam3 did not amplify in any sample from this site. We used linear mixed models to test for differences in genetic diversity between sites, with site as a fixed effect, and locus as a random effect due to inter-marker variation in diversity (Soro et al. 2017; Maebe et al. 2018). We tested both rarefied allelic richness and H_S in separate models (8 loci datasets), and ran the models using the R package 'lme4' (Bates et al. 2015). We used likelihood ratio tests to compare these models against their respective null models (i.e. excluding the fixed effect factor 'site'). When significantly different, we ran a post-hoc Tukey test to determine which sites significantly differed using the *glht* function in the R package 'multcomp' (Hothorn et al. 2008).

We tested for genetic signatures of bottlenecks at each sampling site by testing for significant heterozygosity excess in relation to allelic richness (Piry et al. 1999), as implemented in INEst v2.1. In bottleneck events, both heterozygosity and the number of alleles reduce; however, the allelic richness declines faster than heterozygosity, resulting in heterozygosity excess. We ran the two-phase mutation model with the proportion of multi-step mutations set as 0.22, and the average multistep mutation size as 3.1,

as recommended by Chybicki and Burczyk (2009). We ran the model using 100,000 coalescent simulations, and tested significance using the Wilcoxon signed-rank test, calculated based on 1,000,000 permutations.

Population structure analysis

The traditional measure of subpopulation differentiation, F_{ST}, can underestimate differentiation when variation in markers is high (Jost 2008). Because high numbers of alleles were present in a number of loci in our samples (up to 97 alleles per locus; Table S2), we also used Jost's *D* to estimate population differentiation (Jost 2008). We calculated population pairwise *D* and F_{ST} in GenoDive using the eight loci dataset (referred to as *D* and $F_{ST\ (8)}$ hereafter), and tested significance using 50,000 permutations (with B-Y correction applied for multiple tests). We also calculated F_{ST} between population pairs for all ten loci with ENA correction for null alleles in FreeNA (referred to as $F_{ST\ (10)}$ hereafter). We tested correlation between matrices of $F_{ST\ (8)}$, $F_{ST\ (10)}$, and *D*, using Mantel tests in the 'ade4' package in R with 9999 permutations.

To visualise genetic distance relationships among sites, we carried out Principal Coordinates Analysis (PCoA) in GenAlEx 6.503 (Peakall and Smouse 2012) using standardised covariance matrices of population pairwise *D*.

We tested for the presence of genetic isolation by distance (IBD) patterns in the data by testing the correlation between pairwise matrices of linearised genetic distances ($F_{ST(10)}$/1-$F_{ST(10)}$ and D/1-D) and the logarithm of oceanographic distances with Mantel tests in 'ade4' in R, using 9999 permutations. We calculated least-cost oceanographic distances among sites (i.e., the shortest path, excluding landmasses) using 'marmap' in R (Pante and Simon-Bouhet 2013). We conducted these tests within the Lesser Antilles (Guadeloupe, Martinique, St. Lucia, St. Vincent, Bequia and Mayreau) and within the Florida Keys.

We used a spatially-explicit Bayesian approach implemented in 'Geneland' v4.0.6 in R (Guillot et al. 2008) to identify the number of population clusters (K) and individual assignment probabilities to those clusters, using the 8 loci dataset. We first ran the model using the uncorrelated allele frequencies, spatial, and null allele models with 1,000,000 iterations, 100 thinning and 500 burn-in. According to the authors' advice, we set the maximum number of nuclei to 1320 (3 x sample size) and the maximum rate of the Poisson process to 440 (1 x sample size), and the spatial uncertainty on coordinates to 0.0005. We carried out 10 independent runs of K from 1 to 19, and checked that clusters were consistent among runs. We then selected the run with the highest posterior probability to estimate allele frequencies and cluster locations to use subsequently in the admixture model. We ran the admixture model using the same parameters as above, and extracted the Q matrix of individual probability assignments

289 to each cluster to build a bar plot in Distruct (Rosenberg 2004). We
290 repeated the analysis on each identified cluster to test for the presence of
291 substructure in a hierarchical clustering approach *sensu* Vaha et al. (2007),
292 modifying the maximum number of nuclei and the maximum rate of the
293 Poisson process according to the number of samples in the data subsets.
294
295 We carried out Discriminant Analysis of Principal Components (DAPC)
296 (Jombart et al. 2010) using 'adegenet' (v2.0.1) (Jombart 2008) in R. This
297 method uses Principal Component Analysis to transform the data, and then
298 uses the retained principal components in Discriminant Analysis. This is
299 effective in minimising within-group genetic variation and maximising
300 between-group variation, and does not make assumptions regarding HWE
301 in populations. The number of principal components retained varied among
302 analyses; if too many are retained, resulting membership probabilities can
303 be unstable. We therefore retained the maximum number possible without
304 compromising stability (displayed graphically in each DAPC plot). As
305 previously, we repeated the analysis for each multi-site cluster identified.
306
307 **Results**
308 **Summary statistics, Hardy-Weinberg equilibrium and F_{IS}**
309 All loci were in linkage equilibrium after B-Y correction ($p > 0.05$). Two
310 identical multilocus genotypes (i.e., clones) were present in the dataset
311 (both individuals from Turneffe Atoll), one of which was removed for
312 subsequent analyses. Average null allele frequencies were high at many

313 loci, overall ranging from <0.001 (Icam32) to 0.323 (Icam34) (Table S2).
314 Loci were highly polymorphic, with total number of alleles per locus ranging
315 from 4 to 97 (Table S2).
316
317 There were significant departures from HWE ($p < 0.05$) at a number of loci
318 and sites following the correction for multiple tests (Table S3). Null allele
319 corrected average F_{IS} values were all positive, ranging from 0.033 in
320 Panama 2 to 0.450 in Bamboo Key, Florida (Table 2); however, posterior
321 95% probability intervals included zero for all sites but Bamboo Key. Model
322 comparison using DIC values revealed that null alleles were important in
323 affecting F_{IS} estimates in all sites, along with genotyping failure and/or
324 inbreeding in some of the populations (Table S4).
325
326 **Genetic diversity and bottlenecks**
327 H_O ranged from 0.305 (Kemp Channel) to 0.565 (Panama C) within sites,
328 and H_S ranged from 0.528 (Gray's Reef) to 0.761 (Mayreau) (Table 2). The
329 linear mixed model showed that site had no significant effect on H_S;
330 however, the p value was marginal ($X^2 = 28.315, p = 0.057$). Rarefied allelic
331 richness per site ranged from 3.258 +/-0.552 SE (Waltz Key) to 4.667 +/-
332 0.589 SE (Mayreau) (Table 2, Fig. S1). Site had a significant effect on
333 rarefied allelic richness according to the linear mixed model ($X^2 = 40.695$, p
334 = 0.001). However, post hoc Tukey tests revealed that only three pairs of
335 sites were significantly different: Allelic richness was significantly higher at
336 St. Lucia than at Turneffe Atoll (z = -4.494, p = 0.049); at Mayreau than at

337 Turneffe Atoll, (z = -3.859, p = 0.013) and at Mayreau than at Waltz Key (z
338 = -3.738, p = 0.022). All other site-by-site comparisons were not significant
339 (p > 0.05), although three comparisons were close to significance (higher
340 allelic richness at St. Lucia than at Waltz Key: z = -3.373, p = 0.072; at
341 Bequia than at Turneffe Atoll: z = -3.421, p = 0.062; and at Bequia than at
342 Waltz Key: z = -3.300, p = 0.083). Rarefied private allelic richness ranged
343 from 0.237 +/-0.107 (Waltz Key) to 1.302 +/-0.432 (St. Lucia) (Table 2, Fig.
344 S1).
345
346 Significant heterozygosity excess with respect to allelic richness was
347 detected only at Bamboo Key (p = 0.014), indicative of a genetic
348 bottleneck.
349
350 **Population structure**
351 Our analyses showed strong population structure across the region, with
352 $F_{ST(10)}$ reaching 0.233 (between Gray's Reef and Panama 1) and D
353 reaching 0.598 (between Gray's Reef and Sapodilla Cayes) (Table S5,
354 Table S6). The genetic distance measures were strongly correlated ($F_{ST\ (10)}$
355 and $F_{ST\ (8)}$: r = 0.921, p < 0.001; $F_{ST\ (8)}$ and D: r = 0.908, p < 0.001; $F_{ST\ (10)}$
356 and D: r = 0.825, p < 0.001).
357
358 IBD was significant within the Lesser Antilles subset (pairwise distances 15
359 – 443 km; $F_{ST(10)}$ r = 0.793, p = 0.001; D: r = 0.688, p < 0.001) (Fig. 2a) but

not within the Florida subset (pairwise distances 10 – 115 km; $F_{ST(10)}$: p = 0.483; D: p = 0.476) (Fig. 2b).

Geneland analysis of the whole dataset identified four genetic clusters (Fig. 3a), with further population structure identified on subsequent hierarchical analysis of the original clusters. Clusters were composed as follows: 1) all USA sites; 2) the southern Lesser Antilles sites (St. Lucia, St. Vincent, Mayreau and Bequia); 3) the Panama, Turneffe Atoll, Guadeloupe and Martinique sites; and 4) the Sapodilla Caye site in southern Belize alone. Subsequent analysis of multi-site clusters identified further substructure between St. Lucia and St. Vincent, Mayreau, and Bequia (Fig. 3b), and between Turneffe Atoll, Panama, Martinique, and Guadeloupe (Fig. 3c), giving a total of 8 clusters over all analyses. The USA sites remained a single cluster.

The DAPC showed a clear separation of the Sapodilla Cayes from all other sites upon analysis of the full dataset (Fig. 4a). In the subsequent analysis excluding the Sapodilla Cayes, sites formed three clusters (Fig. 4b), comprising: 1) all USA sites; 2) St. Vincent, Mayreau, Bequia, St. Lucia and Martinique; and 3) Panama, Turneffe Atoll and Guadeloupe (Fig. 4b). DAPC analyses conducted on these clusters revealed further substructure (Fig. 4c-e): Panama, Guadeloupe and Turneffe Atoll separated into individual groups (Fig. 4c); Bequia, Mayreau and St. Vincent clustered together, while St. Lucia, Martinique 1 and Martinique 2 formed separate

384 but closely-located clusters (Fig. 4d). In common with other analyses, the
385 USA sites did not separate into multiple clusters, although Lakes Passage
386 was the most differentiated (Fig. 4e).
387
388 PCoA plots showed some regional-scale clustering patterns consistent with
389 the Geneland and DAPC analyses (Fig. 5). The first axis represented
390 25.62% of the variation in *D* among sites, the second axis represented
391 21.02%, and the third axis represented 12.48%, giving a total of 59.12%
392 over the three axes. The first axis separated the USA sites from the rest of
393 the sites, while the second axis clearly separated a St. Vincent, Mayreau
394 and Bequia cluster (Fig. 5a). The third axis separated the Sapodilla Cayes
395 from all other sites (Fig. 5b).
396
397 **Discussion**
398
399 **Departures from Hardy-Weinberg equilibrium and null alleles**
400 We observed significant deviation from HWE at many loci and sites. There
401 are many potential biological causes of this, including inbreeding, which
402 increases population homozygosity. In sponges, inbreeding could result
403 from low dispersal of planktonic sperm and larvae, which can lead to
404 philopatry and higher incidences of non-random mating.
405
406 Another possible cause of HWE departure is genetic structure within a
407 population, or set of samples, known as the Wahlund effect. In a single

408 geographical site, this could be caused by stochasticity, variation in
409 selective pressures, and changes in ocean circulation that alter dispersal
410 patterns over time (Tesson et al. 2014). Although samples were collected
411 over a three-year period, we do not believe this caused temporal structure
412 in our dataset, as sponges are sessile and slow growing. The high
413 polymorphism of the microsatellite loci used in this study may have also
414 contributed to HWE deviation, as HWE tests are very sensitive to
415 individuals homozygous for rare alleles (Morin et al. 2009).
416
417 Deviation from HWE can also be caused by null alleles, which were indeed
418 found at the majority of loci. Null alleles increase estimates of
419 homozygosity, subsequently raising F_{IS} values. In addition, null alleles can
420 artificially increase F_{ST} values and estimates of population differentiation
421 (Chapuis and Estoup 2007). To mitigate these effects as far as possible,
422 we corrected F_{ST} values and population average F_{IS} values for null alleles,
423 we used the null allele model in Geneland, and we excluded the loci that
424 contributed substantially to F_{ST} skew from other analyses.
425
426 Both HWE deviations and null alleles are common in sponge microsatellite
427 studies (Dailianis et al. 2011; Chaves-Fonnegra et al. 2015; Pérez-Portela
428 et al. 2015; Giles et al. 2015; Guardiola et al. 2016; Richards et al. 2016),
429 suggesting that common life history trends could contribute to these
430 observations. High F_{IS} and HWE departures are common among marine
431 invertebrates with free-spawned planktonic sperm. Addison and Hart (2005)

proposed that this could be associated with higher levels of null alleles, due to higher numbers of cell cycles for sperm production causing increased mutation rates (though sperm production varies interspecifically, and is unknown for *I. campana*).

Regional-scale population structure

Our results show the presence of strong regional population structure in *I. campana* in the Greater Caribbean, with a total of eight to nine genetic clusters identified, indicating highly differentiated populations across the region. This agrees with studies of the Caribbean sponges *C. delitrix* (using microsatellites; Chaves-Fonnegra et al. 2015) and *C. vaginalis* (using mtDNA and nuclear genes; DeBiasse et al. 2016), supporting that the short-lived nature of sponge larvae constrains dispersal, and is an important driver of population structure across the phylum.

The *I. campana* population in the Sapodilla Cayes was genetically distinct from all other sites according to the DAPC and Geneland analyses. In addition, *D* values were almost always higher for all population pairwise comparisons involving this site than for any other population pairs. The Sapodilla Cayes are situated in the south of the Mesoamerican Barrier Reef System (MBRS), an area subject to highly retentive oceanographic conditions (Martínez et al. 2019): a weak southward flowing coastal current and the anti-clockwise Gulf of Honduras Gyre (Ezer et al. 2005; Carrillo et al. 2015), contrasting with the northerly flowing Yucatan Current in the north

MBRS. Furthermore, river discharge into the Gulf of Honduras may form an additional barrier to dispersal for stenohaline marine larvae, such as *Ircinia* spp. (Soto et al. 2009). Genetic studies show population differentiation between the north and south MBRS in the corals *Montastrea annularis* (Foster et al. 2012) and *Orbicella faveolata* (Rippe et al. 2017), as well as the neon goby *Elacatinus lori* (D'Aloia et al. 2017). Genetic evidence also suggests that the lobster *Panulirus argus* experiences higher self-recruitment in the south (Truelove et al. 2014). In addition, Muhling et al. (2013) found distinct larval fish assemblages in the north and south MBRS. The breadth of taxa that are affected by this divide, with their varied life histories, supports that ocean circulation is driving differentiation in this area.

The high level of genetic differentiation found at the Sapodilla Cayes in *I. campana* may be a case of cryptic speciation, as suggested for the neon goby *E. lori* in the same region (D'Aloia et al. 2017). Cryptic species in sponges are common due to absences in morphological variation caused by a lack of complex morphological traits, as well as phenotypic plasticity or convergent evolution resulting in similar morphologies (Sole-Cava et al. 1991; Xavier et al. 2010). However, further studies using phylogenetically-informative markers are needed to explore this possibility further in *I. campana*.

Excluding the Sapodilla Cayes, the remaining sites split into three main clusters in both the DAPC and Geneland analyses prior to further hierarchical analyses. The composition of clusters was consistent among analyses except for the Martinique sites, which clustered with Turneffe Atoll, Panama and Guadeloupe in the Geneland analysis, and with Bequia, Mayreau, St Vincent and St. Lucia in the DAPC. However, cluster-based models are known to not perform as well when genetic variation does not fall into discrete groups, but follows a strong IBD pattern, as observed in the Lesser Antilles (Guillot et al. 2005).

The cluster formed by Turneffe Atoll, Panama and Guadeloupe (and Martinique in the Geneland analysis) is unexpected given the genetic and geographic distances among these locations, and the absence of specific water circulation patterns that might cause such a grouping. However, when analyses were repeated including only these sites, the sites divided into three separate clusters. This demonstrates the utility of repeating analyses on multi-site clusters to uncover further patterns of population structure that are not revealed in overall dataset analysis (Janes et al. 2017). The preliminary grouping of these sites may be partially due to size homoplasy in the microsatellites, which occurs when different alleles are identical lengths, and are consequently scored as the same allele – potentially creating spurious links (Estoup et al. 2002).

502 The sites in the USA formed a single cluster, comprising the Florida Keys
503 sites and Gray's Reef National Marine Sanctuary, despite a distance of
504 ~770 km separating these areas, and the moderate F_{ST} and D values.
505 Although direct larval transport between the areas is very unlikely, indirect
506 connectivity could be maintained via larval transportation in the Florida
507 Current, coupled with "stepping stones" of suitable coastal habitat
508 harbouring intermediate populations along the south eastern coast of
509 mainland USA. The USA cluster is probably distinct from the other
510 Caribbean sites sampled due to distance rather than any particular
511 oceanographic barrier; further fine-scale sampling of sites in the Greater
512 Antilles and Gulf of Mexico would be required to explore this further.
513
514 **Population structure at smaller spatial scales**
515 The Mantel test indicated strong IBD within the Lesser Antilles (where
516 distances between sites ranged 15 – 443 km). IBD is driven by distance-
517 limited dispersal (Wright 1943; Aguillon et al. 2017), which is consistent
518 with the short planktonic duration common for lecithotrophic sponge larvae.
519 IBD patterns were also observed in the coral *A. palmata* in this area
520 (Japaud et al. 2019), and have been found in other sponges at similar
521 spatial scales elsewhere (Bell et al. 2014; Pérez-Portela et al. 2015; Riesgo
522 et al. 2016). However, many sponge studies have found IBD to be absent
523 or very weak, with oceanographic conditions forming better predictors of
524 population structure (Giles et al. 2015; Taboada et al. 2018; Riesgo et al.
525 2019). This suggests that when oceanographic barriers are absent, limited

larval dispersal in sponges can cause distance-decay relationships in genetic structuring.

In contrast, genetic IBD was not significant in the Florida Keys archipelago, where distances between sites ranged 10 - 115 km. Pairwise genetic differentiation between sites in Florida were relatively low (null allele corrected F_{ST} ranged between 0.006 and 0.072), and the sites formed a single cluster in Geneland, PCoA and DAPC analyses. This suggests that larvae disperse across the area with sufficient regularity to maintain gene flow (although genetic similarity does not exclude the possibility of recent divergence). This result is interesting given the short larval duration and low dispersal capacity predicted for sponges, but concurs with other studies that show higher than expected levels of connectivity and dispersal (Chaves-Fonnegra et al. 2015; de Bakker et al. 2016). In other sponges, stronger genetic structure has been observed (e.g., Riesgo et al. 2019), most strikingly on spatial scales of centimetres to tens of metres in *Crambe crambe* and *Scopalina lophyropoda* (Calderón et al. 2007; Blanquer et al. 2009). This variation could be due to larval characteristics (Uriz et al. 2008); compared to other sponges, Irciniidae larvae are relatively strong swimmers with larger lipid stores (Ereskovsky and Tokina 2004; Mariani et al. 2006), theoretically aiding dispersal.

Although the Florida Keys formed a single genetic cluster, 8 out of 15 pairs of sites showed significant but low genetic differentiation, with no site

consistently emerging as different from the rest – a common pattern in marine systems termed ‘chaotic genetic patchiness’ (Johnson and Black 1982). Chaotic genetic patchiness with weak or no IBD has also been found in other sponges across the Florida reef tract (DeBiasse et al. 2010; Chaves-Fonnegra et al. 2015; Richards et al. 2016), and in a co-occurring sponge, *Spheciospongia vesparium* (Griffiths et al. 2020), which shared many sampling sites with this study.

Chaotic genetic patchiness can potentially result from a number of different processes (Eldon et al. 2016). One proposed cause is the random survival of larval cohorts due to stochastic oceanographic conditions, found in species with high fecundity and high larval mortality, termed ‘sweepstakes reproductive success’ (Hedgecock and Pudovkin 2011; Jolly et al. 2014). Another possible cause of temporal variation in recruitment and dispersal dynamics could be variability in local hydrodynamics across time (Schunter et al. 2019). Water circulation is highly variable among shallow, nearshore areas in Florida due to the predominance of wind-driven currents and storms regularly altering bathymetry. Asynchronicity in reproduction within populations could be a further component of temporal variability in recruitment dynamics (Eldon et al. 2016). Another possible cause is differential post-settlement selection (Norderhaug et al. 2016); in *I. campana* this could result from high selection pressure exerted by cyanobacterial blooms (Butler et al. 1995) or disease (Maldonado et al. 2010). Indeed, we found evidence of a genetic bottleneck at Bamboo Key

574 following a known mass mortality caused by a cyanobacterial bloom (see
575 section below).
576
577 In Martinique, genetic differentiation was higher than expected between the
578 two sampling sites (F_{ST} = 0.063) considering their proximity (15 km). In
579 addition, the DAPC and PCoA analyses highlighted genetic separation
580 between the sites. Diamond Rock (MAR2) is a small island located three
581 km off the Martinique coast. Both the island and the channel separating it
582 from the mainland are locally known to experience strong currents (pers.
583 comm. G. Tollu), and early modelling of water circulation suggests the area
584 may be influenced by a small gyre off the southern coast of Martinique
585 (Lazure et al. 1996), which could reduce connectivity between the sites.
586 However, more in-depth work on local water movement patterns is needed
587 to further interpret the cause of this genetic differentiation.
588
589 **Genetic diversity and bottlenecks**
590 We found evidence of a genetic bottleneck in Bamboo Key, where previous
591 mass mortalities were observed as part of widespread, reoccurring mass
592 mortalities in sponge communities across the Florida Keys associated with
593 cyanobacterial blooms (Butler et al. 1995; Stevely et al. 2010). This
594 prompts concern for the population's resilience and adaptability to future
595 stressors (Wernberg et al. 2018). Bottleneck signatures have also been
596 found in other sponges in Mediterranean sites due to overharvesting
597 (Pérez-Portela et al. 2015), and disease (Riesgo et al. 2016), underscoring

598 the risks to genetic diversity levels for sponge populations that undergo
599 significant declines. However, as null alleles can affect estimates of both
600 heterozygosity and allelic richness, the possibility of a false positive at
601 Bamboo Key cannot be excluded, given the presence of null alleles in our
602 dataset.
603
604 Past mortalities also occurred in another of our sampling sites (Long Key in
605 Florida), but a bottleneck signature was not detected here. This could be
606 because any loss in genetic diversity was rapidly regained through
607 recruitment and gene flow from other areas. Indeed, the Long Key site is
608 dominated by long-shore currents whereas the Bamboo Key site sits within
609 a bay subject to a local gyre. Alternatively, it is possible that the Long Key
610 result is a false negative, as heterozygosity excess tests are sensitive to
611 mutation model selection and sample size limitations (Garza and
612 Williamson 2001; Peery et al. 2012). However, we found genetic diversity
613 was generally high in *I. campana*, with similar H_S ranges to other sponges
614 (Chaves-Fonnegra et al. 2015; Giles et al. 2015; Richards et al. 2016;
615 Riesgo et al. 2019); furthermore, allelic richness and H_S in Long Key were
616 not significantly different from other sites.
617
618 Rapid population declines have not been reported in any of our other study
619 sites, nor were significant bottleneck signatures detected. In addition,
620 genetic diversity was largely similar across sites, with only Waltz Key and
621 Turneffe Atoll showing significantly lower allelic richness than some Lesser

622 Antilles sites (Mayreau, St. Lucia and Bequia). However, we did observe a
623 disease-like condition in individuals harbouring necrosis of various stages
624 in Guadeloupe and Bequia during sampling for this study, and on other
625 occasions in Martinique (pers. obs. T. Pérez and S. Griffiths). Indeed,
626 disease outbreaks and mass mortalities have been reported multiple times
627 in *Ircinia*, often linked with higher temperatures (Perez et al. 2000;
628 Maldonado et al. 2010; Stabili et al. 2012; Riesgo et al. 2016). Given this
629 vulnerability, and the bottleneck signature found in Bamboo Key, continued
630 genetic monitoring of *I. campana* would be prudent to ensure bottlenecks
631 are accurately identified for effective management of the species (Schwartz
632 et al. 2007).
633
634 **Conservation implications**
635 Understanding the scale and magnitude of connectivity among populations
636 is important for the management and conservation of marine ecosystems
637 (Almany et al. 2009). Firstly, our results contribute to the increasing
638 evidence suggesting that, for a number of taxa, the Sapodilla Cayes
639 experiences low connectivity with other areas, and relies on high self-
640 recruitment. This supports the current protection status for this area (the
641 Sapodilla Cayes Marine Reserve), which is important to bolster the
642 resilience of the population at this location, and to protect its unique genetic
643 diversity. This study also implies that this population may form a separate
644 management unit from other Belizean Marine Protected Areas (MPA),
645 however, more extensive sampling of Belizean MPAs would be required to

explore this further. More generally, our results tentatively suggest that to form connected MPAs for this species, protected areas would need to be situated within ~50-100 km of each other, where suitable habitat exists and oceanographic barriers are absent. This would allow sufficient spillover of larvae to non-protected areas and would maintain connectivity between protected areas.

Our results also provide insight for management of mass mortality-affected sites in Florida. Restoration through fragmenting and transplanting healthy sponges has been successfully used to repopulate barren areas (Butler et al. 2016). Our results suggest that gene flow occurs over the length of the Keys, indicating that donor sponges may be sourced from any area of the Keys without risk of outbreeding depression. Our results also indicate that connectivity over the area is unpredictable; therefore, restoration is supported as an important strategy to ensure rapid repopulation of mass mortality affected areas. Genetic diversity is naturally high in *I. campana* populations; this should be maintained in restored sites through the use of a large number of donor sponges, rather than extensive fragmentation of few sponges.

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Conflict of Interest

The authors declare no conflict of interest.

Data Archiving

693 Microsatellite genotype data are available at
694 https://doi.org/10.5061/dryad.gtht76hh7.
695

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983 **Figure legends**

Figure 1: Map showing *Ircinia campana* sampling locations. GR: Gray's Reef; LK: Long Key; BK: Bamboo Key; KC: Kemp Channel; WK: Waltz Key; BC: Boca Chica Channel; LP: Lakes Passage; TA: Turneffe Atoll; SC: Sapodilla Cayes; PAN1: Panama 1; PAN 2: Panama 2; PAN 3: Panama 3; MAY: Mayreau; BEQ: Bequia; STV: St. Vincent; STL: St. Lucia; MAR1: Martinique 1; MAR2: Martinique 2; GU: Guadeloupe.
Inset (bottom left): *Ircinia campana* photographed in Bequia (T. Pérez). Arrows show major ocean current patterns in the Caribbean Sea. Basemaps: ESRI, Natural Earth.

Figure 2: Genetic isolation by distance in *Ircinia campana*, showing site pairwise linearised F_{ST} values and logarithm of oceanographic distances a) within the Lesser Antilles; and b) within the Florida Keys.

Figure 3: Geneland plot showing genetic clusters and individual admixture proportions in *Ircinia campana.* Each bar represents an individual; colours represent genetic cluster identity, and bar heights represent inferred membership proportions to genetic clusters. The analysis was firstly carried out over all sites, showing K = 4 genetic clusters (a); separate analyses were subsequently conducted on multi-site clusters identified therein (b, c), showing K = 4 (b) and K = 2 (c) genetic clusters. Analysis on the USA cluster yielded K = 1 (not shown).

Figure 4: Discriminant analyses of principal components (DAPC) of *Ircinia campana.* Individual points represent genotyped individuals; inertia ellipses summarise the point cloud for each site. Sequential analyses were carried out in a hierarchical approach by repeating analyses on clusters detected (a – e). Insets show the proportion of principal component eigenvalues and discriminant analysis eigenvalues retained.

Figure 5: Principal coordinates analysis (PCoA) using site pairwise genetic distances (Jost's *D*) calculated using 8 loci among *Ircinia campana* sampling sites.

Tables

Table 1: *Ircinia campana* sampling locations. n = number of individuals successfully genotyped.

Table 2: Genetic diversity and average inbreeding coefficient in *Ircinia campana* per location.