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NEWS AND VIEWS

COMMENT

MOLECULAR ECOLOGY WILEY

Cryptic lineages respond differently to coral bleaching

Abstract

Coral cover is decreasing worldwide largely as a result of a rise in seawater temperatures that triggers coral bleaching and induces coral mortality. How coral reefs will respond to climate change will be a function of genetic variation and how it is partitioned within and among species. A critical initial step is to accurately delineate species and quantify their physiological potential to cope with heat stress. Cryptic species are morphologically similar but genetically distinct and may respond physiologically differently to climate change. A dominant Caribbean reef builder severely affected by climate change is the mountainous star coral, *Orbicella faveolata*. Recently in this journal, Dziedzic et al. reported quantitative genetic variation in the physiological response to thermal stress in a single population of this species, suggesting that variation within populations will allow these corals to adapt to rising ocean temperatures. We reanalysed their data and found multiple cryptic lineages rather than a single panmictic population, with one of the lineages being heat-intolerant. While different cryptic lineages co-occur in certain locations, there is at least one lineage that occurs only in a single location. Our finding of hidden lineages within a threatened species highlights the varying extinction risks faced by these independently evolving groups, especially when the prospects of survival under warmer oceans seem favourable for only some of them.

1 | INTRODUCTION

Rising seawater temperatures are decimating tropical and subtropical shallow Scleractinian (i.e., hard corals) reefs worldwide (Hughes et al., 2018; Smale et al., 2019). How reef ecosystems will respond to warmer oceans will be a function of genetic variation and how it is segregated among populations and communities. A critical priority is to understand differences in physiological tolerance among populations to identify the functional diversity within and among species that are available to cope with warmer oceans (Edmunds et al., 2014; van Woesik et al., 2011). To understand how reef species will fare under warmer oceans and design successful management strategies, it is critical to accurately delineate species (or independently evolving lineages) as it will allow us to (a) study the physiological significance of genetically distinct but morphologically similar groups within species complexes, (b) properly estimate the relative abundance of each cryptic species, and (c) accurately quantify population sizes and genetic connectivity among populations, and establish whether many cryptic lineages drive ecosystem-level changes with narrow distributions or a few species with independent demographics.

The potential for cryptic species, morphologically similar yet genetically distinct groups (Bickford et al., 2007; Knowlton, 1993), remains an overlooked aspect of coral reef biology, even within common reef dwellers (Prada & Hellberg, 2013; Prada et al., 2008, 2014; Rosser, 2015; Warner et al., 2015). Morphological and traditional molecular identification of corals do not always reflect the true identity of genetic species, owing to different rates of evolution for both types of characters, phenotypic plasticity, convergent evolution and hybridization (Budd et al., 2010; Willis et al., 2006). Such hidden taxonomic diversity frequently harbours distinct physiological variation and allows cryptic lineages to occupy heterogeneous habitats and respond differently to climatic variation (Struck et al., 2018). Failure to recognize the different susceptibilities of cryptic species to climate change stressors can underestimate threats to local populations, and lead to biodiversity losses (Fišer et al., 2018). Therefore, uncovering cryptic diversity stands as a major research priority to account for ecosystem dynamics and for forecasting future states (Bálint et al., 2011). The advent of genome-wide data has facilitated the detection of such diversity, a task that thus far has not always been achievable with traditional molecular markers alone, such as single genes or microsatellites (Leaché & Oaks, 2017).

A key Caribbean reef builder severely affected by climate change is the mountainous star coral, *Orbicella faveolata*. This species was once part of the *Montastraea* (now *Orbicella*) *annularis* species complex. While originally deemed as a single cosmopolitan species with different ecotypes (Goreau, 1959; Graus & Macintyre, 1982), members of this complex were later described as three different species (Weil & Knowton, 1994) with clear differences in ecology (Pandolfi & Budd, 2008), morphology (Budd & Klaus, 2001; Pandolfi & Budd, 2008), spawning behaviour (Knowlton et al., 1997; Levitan et al., 2004), gamete compatibility (Fogarty et al., 2012; Levitan et al., 2011) and genetics (Fukami et al., 2004; Levitan et al., 2011). More recently, this widely distributed Caribbean coral species was taxonomically revised and assigned to the genus *Orbicella* and the family Merilunidae, along with *O. annularis* and *O. franksi* (Budd et al., 2012).

Typically found between 1 and 50 m depth, depending on reef features, this broadcast spawning coral associates with endosymbiotic dinoflagellates (Symbiodiniaceae) from multiple genera across locations and depths on tropical Atlantic Western reefs (DeSalvo et al., 2010; Thornhill et al., 2009). Currently placed under threatened status (NOAA, 2014), this massive coral has served as a model species for numerous physiological studies across the Caribbean (Colombo-Pallotta et al., 2010; DeSalvo et al., 2010; Kemp et al., 2015) as well as for understanding the genetic basis of adaptation to higher temperatures and bleaching (Manzello et al., 2019; Wright et al., 2019).

A recent study in this journal (Dziedzic et al., 2019) found quantitative genetic variation for the physiological response to thermal stress among individuals of *O. faveolata* across four reefs in Bocas del Toro, Panama. The authors concluded, from narrow-sense heritability analyses, that the differential response to bleaching of corals stems from additive genetic variation within a panmictic population. Consequently, they concluded that there is substantial genetic variation to adapt to rising ocean temperatures within a single population of this species. Here, we reanalysed their data and argue that their study is more consistent with the presence of multiple cryptic lineages, some of which are heat-tolerant, rather than as a result of diversity within a single species.

2 | MATERIALS AND METHODS

To test whether genetic variation in *Orbicella faveolata* from Bocas del Toro represents multiple cryptic lineages (rather than a single species), we carried out seven analyses using the original data from Dziedzic et al. (2019): (a) a Discriminant Analysis of Principal Components (DAPC), (b) a Bayesian clustering method, (c) a neighbour-joining (NJ) distance tree based on the UPGMA algorithm, (d) a maximum-likelihood (ML) phylogenetic tree, (e) a species tree under the Bayesian multispecies coalescent framework of SNAPP, (f) a Symbiodiniaceae community taxa prediction from ITS2 under the SYMPORTAL analytical framework and (g) a Bayes factor contingency table to associate lineage assignment with sampling location and algal symbiont type.

Multilocus raw sequence data (SRA: BioProject PRJNA413258) from 39 samples were downloaded from the National Center for Biotechnology and Information (NCBI) server. These colonies corresponded to seven shallow (10 m) sampling sites at four islands across the Bocas del Toro Archipelago (Isla Colon, Isla Solarte, Isla Cristobal and Isla Bastimentos). We mapped the genomic data to a reference genome (Prada et al., 2016) and scored 383,160 single nucleotide polymorphysms (SNPs) following the *dDocent* pipeline (Puritz et al., 2014). We removed all indels, retaining only biallelic SNPs that were genotyped in at least 70% of individuals, had a minor allele frequency of 0.05 and had a minimum mean coverage of 20×. To reduce linkage disequilibrium, we kept only SNPs at least 1,000 bp apart. Remaining loci were screened for statistical outliers potentially under strong selection using BAYESCAN (Foll, 2012). Our final data set consisted of 3,560 high-confidence SNPs. All filtering steps were done using VCFTOOLS (Danecek et al., 2011). We then identified potential clones using the R package POPPR (Kamvar et al., 2014).

Initially we examined genome-wide variation with DAPC using the R package ADEGENET (Jombart, 2008; Jombart et al., 2010). We estimated the number of clusters (from 1 to 8) using *find.clusters* in ADEGENET and selected the optimal number of groups along using a Bayesian Information Criterion (BIC) approach. To avoid overfitting, we used the *optim.a.score* function to determine the number of principal component (PC) axes to be retained. We then used a Bayesian clustering method as implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) to infer the number of genetic clusters (from 1 to 8) and potential admixture. Structure was performed on unlinked SNP data sets and run in parallel using STRAUTOPARALLEL version 1.0 (Chhatre & Emerson, 2017) using an admixture model with correlated allele frequencies, and a low ALPHA value (0.1), as recommended for unbalanced sampling schemes (Wang, 2017). Burnin was set to 250,000 followed by 500,000 Markov chain Monte Carlo (MCMC) generations. We evaluated the optimal *K* in STRUCTURE HARVESTER (Earl, 2012) following Evanno et al. (2005). STRUCTURE results were plotted in STRUCTURE PLOT (Ramasamy et al., 2014). Additionally, we estimated the population fixation index (F_{ST}) among the inferred clusters via VCFTOOLS.

To further test for the presence of multiple cryptic lineages, we used an NJ tree based on the UPGMA algorithm using the pairwise genetic distance matrix of genotypes with the R package APE (Paradis & Schliep, 2019), with 1,000 bootstrap replicates to assess branch support. We then built an ML species tree in RAXML-NG (Kozlov et al., 2019), using the GTR + gamma model of sequence evolution, and node support was evaluated with a maximum of 1,000 bootstrapping replicates (autoMRE, cutoff = 0.03).

To test for the number of lineages present in our data set, we used SNAPP in combination with BIC and maximum clade credibility analysis. We first inferred the species tree under the Bayesian multispecies coalescent framework of SNAPP version 1.3 (Bryant et al., 2012) implemented in BEAST2 version 2.5 (Bouckaert et al., 2018), with a path sampling of 24 steps (MCMC length = 1,000,000, preburnin = 1,000). The SNAPP data set consisted of individuals without any missing data, and unlinked biallelic SNPs only. The clusters identified by the genetic-clustering methods (DAPC, NJ and STRUCTURE) were used for clade assignments with no outgroup (Kornilios et al., 2019). Because SNAPP is computationally intensive (our analysis took 1 month), groups included only three to five individuals, for a total of 12 individuals. Marginal likelihood estimates were obtained for each different model run. Each species delimitation model was then ranked by their Marginal Likelihood Estimate (MLE) and Bayes Factors (BFs) following Leaché et al. (2014). BFs were calculated by subtracting the MLE values for two models and multiplying the difference by two (BFD = 2 × (model1 − model2). Log files were combined using LOG COMBINER version 1.1 and input into TRACER version 1.6 (Rambaut et al., 2018). Convergence and estimated sample size (ESS) >200 were assessed using TRACER after a 10% burnin. A maximum clade credibility tree was generated with TREE ANNOTATOR version 2.3

(Bouckaert et al., 2018). Both the consensus tree and all tree topologies were drawn in DENSITREE version 2.2 (Bouckaert, 2010).

To associate algal symbionts with coral lineages, we downloaded the ITS2 amplicon sequencing data from NCBI (SRA: BioProject PRJNA413258). We analysed ITS2 data through SYMPORTAL (Hume et al., 2019), based on the identification of within-sample intragenomic variants of this multicopy marker. This analytical framework outputs ITS2-type profiles representative of putative Symbiodinaceae taxa. Lastly, to test the null hypothesis of no relationship between cryptic lineages and location, as well as ITS2-symbiont profile, we performed a contingency table BF test (Morey & Rouder, 2015). We used the R package BAYESFACTOR under an independent multinomial distribution, and estimated the difference in probability of colonies being found on a given location or associated with a particular symbiont, given their cryptic lineage.

3 | RESULTS

After removing four potential clones from the data set (Figure S1), our NJ tree based on bi-allelic, neutral (no outlier loci detected by BAYESCAN) and unlinked SNPs suggests the presence of three genetically well-supported clades (Figures 1 and 2), henceforth referred to as PAN_1, PAN_2 and PAN_3. We recovered the same three divergent clades using DAPC (Figure 2; Figure S2), while STRUCTURE grouped PAN_1 and PAN_2 as a single cluster with a weak admixture signal between them and PAN_3 (Figure 2; Figure S2; Table S1). The F_{ST} among these three clades ranged between 0.19 (PAN_1 vs. PAN_3), 0.44 (PAN_2 vs. PAN_3) and 0.26 (PAN_1 vs. PAN_2).

The maximum clade credibility tree from SNAPP and BF (Table S2) test also supported the presence of three independent clades (Figure 3) with the same topology as the NJ tree. Likewise, the ML tree recovered the above-mentioned three groups (Figure S4). Importantly these different clades differ physiologically and responded differently to bleaching stress (Figure 4).

We found co-occurrence as well as segregation of lineages across locations. Two lineages co-occurred in at least two locations with PAN_1 present in all locations, and co-existing with PAN_2 at Isla Colon and Isla Solarte. PAN_3 exclusively inhabited Isla Colon (Table S3 and Figure S5), which suggests that this clade may be more geographically restricted (BF = 22.19; Table S4 and Figure S6).

Finally, to characterize the microalgal symbiotic community and its distribution among cryptic lineages, we used the analytical framework of SymPortal. A total of 56 ITS2-defining intragenomic variants (IDVs) were recovered from 35 samples, from which eight ITS2-type profiles for 32 colonies were inferred (Figure S5). Most samples (14) harboured the genus/species *Symbiodinium fitti* (A3), followed by *Breviolum minutum* (B1) with 10 colonies, *Cladocopium* (C3/C7) with five individuals and *Durisdinium trenchiii* (D1) with three samples, and three samples with unnamed/classified variants (five, six and 17 colonies). Although we did not find support for the alternative hypothesis of association between coral lineages and algal symbionts (BF = 2.01; Table S4), some differences in prevalence exist (Figure S6). PAN_1 and PAN_2 are highly promiscuous, associating with taxa from four genera, while PAN_3 seems to associate only with *S. fitti* (Figure S7).

4 | DISCUSSION

Our re-analyses of Dziedzic et al.'s (2019) data suggest that *Orbicella faveolata* from Bocas del Toro Reefs is not a single cosmopolitan species but rather is composed of multiple cryptic lineages with independent evolutionary trajectories. Congruence among delimitation

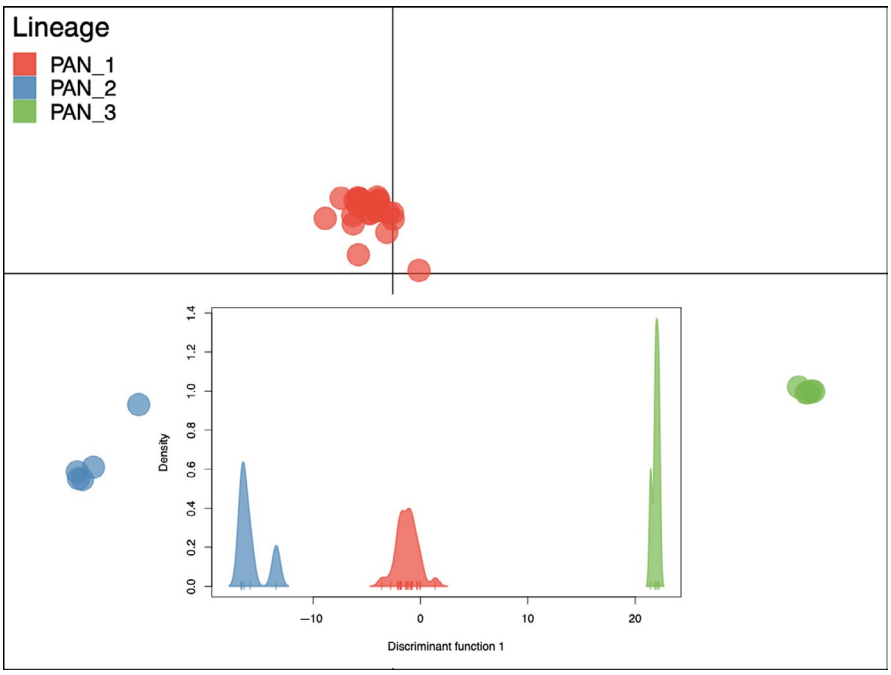

FIGURE 1 Discriminant analysis of principal components (DAPC; optimal $k = 3$ and number of PC = 2). Note the full segregation of the data, suggestive of nonrandomly reproducing groups

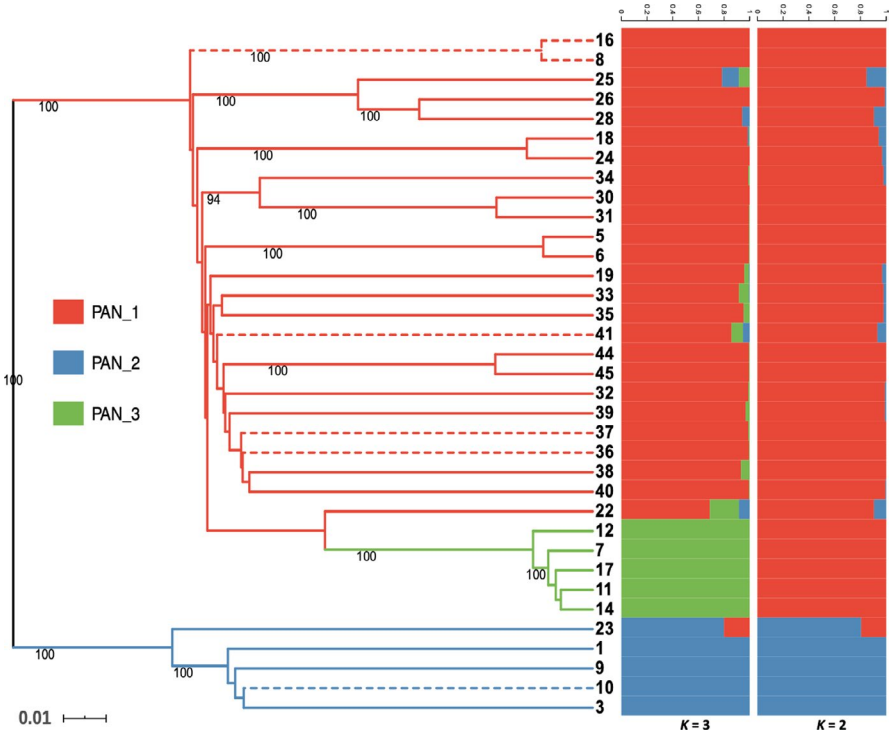

FIGURE 2 Neighbour-joining (NJ) tree using the pairwise genetic distance matrix (node numbers are bootstrap support values), depicting the groups inferred from the genetic clustering methods (dashed branches represent heat-tolerant samples). Hierarchical Bayesian population clustering with STRUCTURE depicting $k = 3$ (optimal) and $k = 4$. Scale bar indicates proportion of loci that are different among individuals

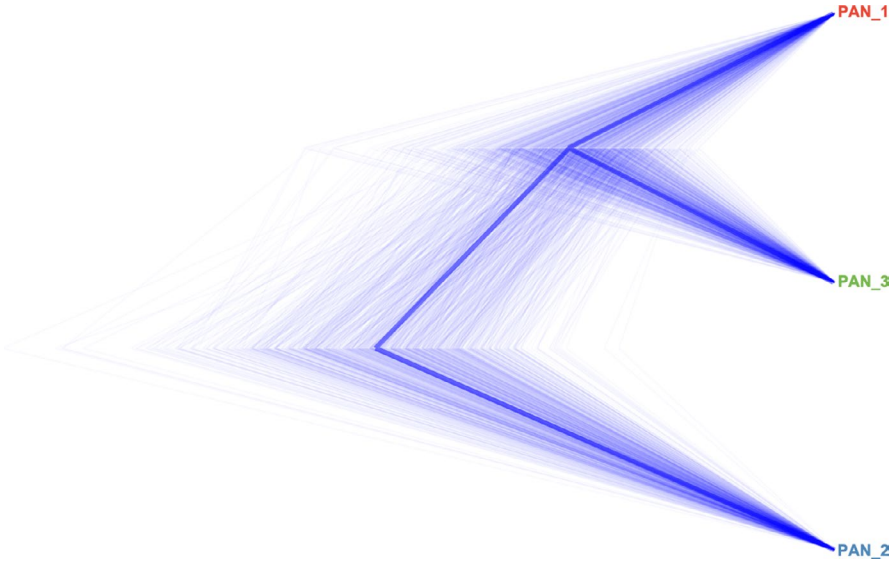

FIGURE 3 Phylogenetic tree inferred from the Bayesian coalescence analysis of SNAPP (the congruent tree is the thick blue line)

methods indicates that at most three cryptic lineages coexist under the nominal species *O. faveolata* in this Caribbean region. Interestingly, thermal tolerance occurs in two of these lineages (PAN_1 and PAN_3), while PAN_2 comprises only heat-sensitive colonies. Despite *Durisdinium* (D1) being typically reported as the most heat-tolerant endosymbiotic partner (Rowan, 2004; Silverstein et al., 2017), symbiont affinity in these three lineages exhibits a promiscuous association, both for susceptible and for heat-tolerant colonies.

Our finding of cryptic lineages falls within the rich history of cryptic species and speciation studies on the *Orbicella* (formerly *Montastraea*) species complex, and it is therefore the first step toward further research to delineate the species boundaries in this radiation using other lines of evidence from reproductive behaviour, morphology and physiology, and to fully test whether these cryptic groups are indeed novel species.

4.1 | Cryptic lineages and thermal tolerance

The presence of heat-tolerant colonies within the PAN_1 and_PAN_3 lineages implies that, under recurrent or stronger bleaching events, the survival of less tolerant lineages, such as PAN_2, will be severely compromised. Given the dramatic pace of the Caribbean reef decline, without a thorough appreciation of the cryptic species composing this pivotal ecosystem, silent diversity losses will take place as we

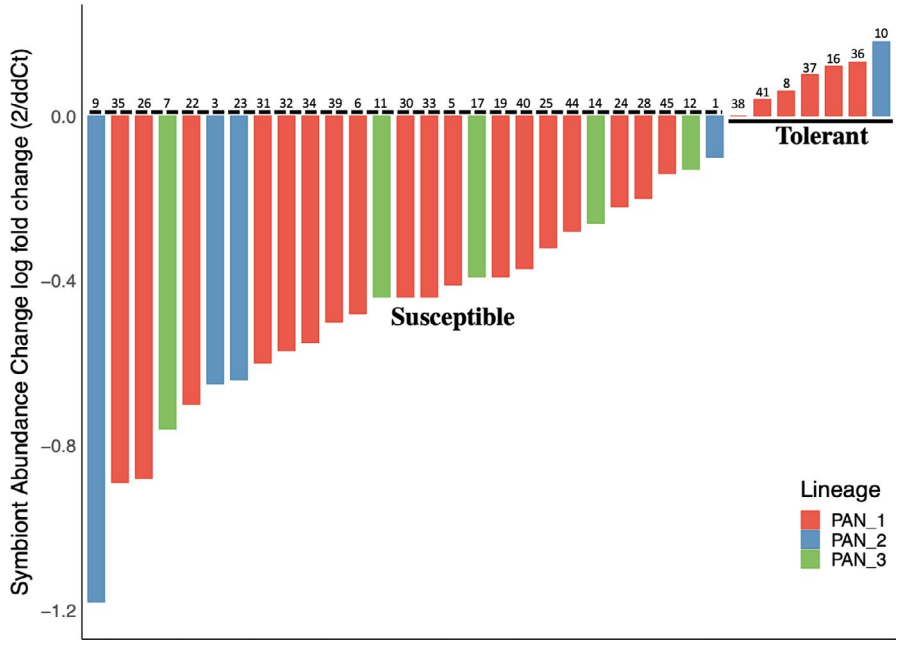

FIGURE 4 Log-fold change (2^{-ddC_T}) algal symbiont abundance per colony using quantitatice PCR after 4 weeks of experimental conditions (modified from Dziedzic et al., 2019). Colours denote the cryptic lineages uncovered in this study. Host cell quantifications (C_T values) were subtracted from symbiont cell quantifications to calculate the dC_T value in each colony, a measure of the ratio of symbiont cells to host cells, for both control and experimental conditions. The dC_T stress value was subtracted from the dC_T control value to generate ddC_T values, representing the symbiont density. Then, Dziedzic et al., used those ddC_T values for each colony to calculate the fold change of symbiont abundance (2^{-ddC_T}), which were then log-transformed to compare across colonies

grapple to capture and protect the true existing biological diversity (Richards et al., 2016). Consequently, a deeper understanding of how cryptic coral lineages vary physiologically is critical to forecasting reef ecosystem composition and resilience. Ecosystem resilience to disturbances hinges not only on individual species trajectories but on the standing community composition under regional conditions and their temporal variability (Edmunds et al., 2014). Therefore, the disappearance of cryptic evolutionary lineages decreases evolutionary potential by disrupting current diversification processes that will impact future biodiversity (Bálint et al., 2011).

As we increase our ability to delineate species using genomic data, we need to accommodate the changes in our understanding that this diversity will bring in terms of species interactions, symbiotic relationships and physiological thresholds to the resilience of reef ecosystems. The future of coral reefs lies largely in their physiological response to heat, which depends directly on their symbiotic algal counterpart. Therefore, having found differential susceptibility to heat among potential cryptic lineages that do not exhibit a discernible affinity for Symbidinaceae taxa, this presents yet another challenge to our understanding of corals' heat tolerance (Kemp et al., 2015; Thornhill et al., 2009, 2014). Although this adds another layer of complexity to coral reef ecology and conservation, it opens up an opportunity for more integrative studies that take into account host cryptic diversity to define whether coral heat tolerance is more or less widely distributed among the most common reef-builder species (Kavousi et al., 2020). Importantly, this would better inform conservation and management decisions because those species thought of as being cosmopolitan might be local or environmentally confined. Endangered coral species, such as *O. faveolata*, would be even at greater peril as their population sizes may have been overestimated.

4.2 | The importance of delineating cryptic lineages to identify the genomic architecture to bleaching

Broadcast spawning corals, such as *O. faveolata*, with larval dispersal capabilities in the order of tens to hundreds of kilometres, have considerable potential for colonizing not only neighbouring but also distant (>100 km) reefs (Davies et al., 2019; Severance & Karl, 2006). In *O. faveolata*, microsatellite loci suggest population connectivity at large spatial (>1,000 km) scales over its entire distribution (Severance & Karl, 2006). A more recent study, however, found population structure among close reefs to be at odds with the predominant ocean circulation patterns in the area (Rippe et al., 2017). This discrepancy might not only reflect inherent difficulties associated with the interpretation and rapid evolution of these markers (Fukami, 2008), but may also suggest the presence of unrecognized diversity, as we have uncovered here.

The co-occurrence of cryptic lineages within Bocas del Toro (<50 km) and even within single reefs suggest reproductive isolation exists among them. We found that PAN_1 occurs across all sampled reefs, PAN_2 co-exists with PAN_1 at two sites and PAN_3 inhabits only one location. In the absence of reproductive isolation and full cross-breeding, genetic differences between

lineages would have been erased via unrestricted gene flow. Even if there is some degree of association of lineages with locations or habitats (as with PAN_3 here), our finding of genetic differentiation at neutral loci across the genome points to a reproductive isolation mechanism that maintains lineages apart even within the crossing range of their gametes (such as across habitats). In fact, cryptic lineages associated with different habitats have previously been reported for reef taxa, in particular across depth gradients (Carlon et al., 2002; Prada & Hellberg, 2013).

It remains to be seen whether the three lineages that we detected here occur across the Caribbean and the Gulf of Mexico, or if they reside in specific habitats at smaller geographical scales (Prada et al., 2008). Similarly, other cnidarians exhibit fine genetic structuring along with restricted geographical areas that are more consistent with the existence of cryptic lineages than within-species genetic diversity (McFadden et al., 2017; Ohki et al., 2015; Richards et al., 2016; Warner et al., 2015). Hence, accurate delimitation of species boundaries is crucial to quantify species diversity and elucidate biologically meaningful patterns of gene flow and dispersal among populations (Prada & Hellberg, 2013; Wham & LaJeunesse, 2016).

Likewise, genome-wide association studies (GWAS), when there is underlying cryptic diversity (stratification), fail to account for the nonindependent distribution of the genetic variation, which is constrained by the particular evolutionary history of each lineage (Sul et al., 2018). In this case, genetic variants found to be associated with thermal tolerance might have been unwittingly conflated with cryptic lineages. We did not pursue a GWAS re-analysis because the small number of individuals composing each cryptic lineage would have further underpowered the inferences made from an already small sample size.

4.3 | Cryptic lineages and symbiotic relationships

Flexible symbiotic partnerships among coral hosts and their algal symbionts underpin dynamic patterns of co-evolution, reflecting multiple prospects for withstanding environmental perturbations, such as those projected under a climate change scenario (Baker, 2003; Voolstra et al., 2011). For hard corals, such flexibility to establish physiological and ecological interactions with their endosymbionts translates directly into unique environmental thresholds given their dependence on symbiosis for energy acquisition (Ziegler et al., 2018). Taxonomic revision of symbiotic microalgae (Symbiodinaceae) found in corals and other invertebrates has revealed that what were once assumed to be single panmictic species encompass multiple genera and species with a more restricted suite of hosts (LaJeunesse et al., 2018; Parkinson et al., 2015; Pochon et al., 2014; Wham & LaJeunesse, 2016). Here, we found large symbiont flexibility in two of the cryptic lineages, whereas only one (PAN_3) had a specific affinity for *Symbiodinium fitti*. In *O. faveolata*, what has been considered as a flexible relationship between a single coral species and multiple symbionts could be more complex given the existence of cryptic coral lineages. Under this view, and in light of our re-analyses, identifying the correct taxonomic units in both hosts and symbionts remains key to understanding the response of coral communities to climate change.

5 | CONCLUSIONS

Our analyses are the first step in a process to fully uncover and describe these cryptic lineages with different levels of physiological heat tolerance. Accurately delineating species, even cryptic ones, is key to reef conservation and management because it allows us to truly estimate the relative abundance and population sizes of these biological units. This, in turn, helps us to understand patterns of gene flow to determine genetic connectivity among populations. Moreover, precise detection and description of cryptic species enhance our ability to establish if ecosystem-level changes are driven by various cryptic lineages that are narrowly distributed, or instead by few species with overlapping or distinct demographics.

Ultimately, whether coral populations can survive to the rapid increase in water temperature while maintaining ecosystem resilience is still under heavy scrutiny. Given the fast decline of coral reefs worldwide, the projected increases in environmental variability, and regardless of the mechanism that corals may have to cope with future changes, identifying evolutionary units of biological diversity is critical to consolidate conservation efforts. Failure to do so, in either host or symbiont, leads to an overestimation of the ecological and physiological ranges of individual species, undermining our view of how reefs will respond to rapidly changing conditions.

AUTHOR CONTRIBUTIONS

M.G-C. and C.P. conceived the study. M.G-C compiled and analysed the data. M.G-C. and C.P. wrote the paper. Both authors read and approved the paper.

DATA AVAILABILITY STATEMENT

Sequence data archived at NCBI's Sequence Read Archive (SRA) BioProject PRJNA413258. Files and scripts used for analysis will be made publicly available at https://github.com/matiasgoco and datadryad.org https://doi.org/10.5061/dryad.9kd51c5f3.

KEYWORDS

coral bleaching, cryptic species, genomics, global warming

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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