Cryptic lineages respond differently to coral bleaching

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Abstract

Coral reefs are losing coral cover across the globe largely as a result of a rise in seawater temperatures that trigger coral bleaching and induce coral mortality. How coral reefs will respond to climate change will be a function of genetic variation and how it is partitioned among species. A critical initial step is to accurately delineate species and quantify their physiological potential to cope with heat stress. Cryptic species, morphologically indistinguishable but genetically different ones, typically harbor distinct physiological variation and respond differently to climatic changes. A dominant Caribbean reef builder severely affected by climate change is the mountainous star coral, *Orbicella faveolata*. Recently, Dziedzic et al. (2019) reported 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 reanalyzed their data and found multiple cryptic lineages rather than a single panmictic population, with only one of the lineages being heat-tolerant. 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 favorable for a few of them only.

Keywords

Coral bleaching, cryptic species, global warming, genomics

Introduction

Rising seawater temperatures are bleaching corals and decimating reefs world-wide (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 available to cope with warmer oceans (Edmunds et al., 2014; van Woesik, Sakai, Ganase, & Loya, 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 1) study the physiological significance of genetically distinct but morphologically similar groups within species complexes, 2) properly estimate the relative abundance of each cryptic species, and 3) accurately quantify population sizes, 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 indistinguishable yet genetically distinct groups (Bickford et al., 2007; Knowlton, 1993), remains an overlooked aspect of coral reef biology, even within common reef dwellers (Prada et al. 2008; Prada & Hellberg 2013; Prada et al., 2014; Rosser, 2015; Warner, van Oppen, & Willis, 2015). Such hidden taxonomic diversity frequently harbors distinct physiological variation and allows cryptic lineages to occupy different habitats and respond differently to climatic variations (Struck et al., 2018). Failure to recognize the different susceptibilities of cryptic species to climate change stressors can underestimate threats to local populations, and ultimately to entire ecosystems (Fišer, Robinson, & Malard,

2018). Therefore, uncovering cryptic diversity stands as a major research priority to account for ecosystem dynamics and forecasting future states (Bálint et al., 2011). The advent of genome-wide data has facilitated the detection of such diversity, a task thus far not always achievable with traditional molecular markers alone (Leaché & Oaks, 2017).

A key Caribbean reef builder severely affected by climate change is the mountainous star coral, *Orbicella faveolata*. Currently placed under threatened status (NOAA & NMFS, 2014), this massive coral has served as a model species for numerous physiological studies across the Caribbean (Colombo-Pallotta, Rodríguez-Román, & Iglesias-Prieto, 2010; DeSalvo et al., 2010) as well as for understanding the genetic basis of adaptation to warmer temperatures and bleaching (Manzello et al., 2018; Wright, Correa, Quigley, & Davies, 2019). A recent study (Dziedzic, Elder, Tavalire, & Meyer, 2019) found variation in the physiological response to thermal stress among individuals of *O. faveolata* across reefs in Bocas del Toro, Panama, which the authors interpreted as indicative of genetic variation within species to adapt to rising ocean temperatures. Here, we reanalyzed their data and find that their study is more consistent with the presence of multiple cryptic lineages (only one of which is heat-tolerant) rather than as a result of diversity within a single species.

Materials and Methods

To test whether genetic variation in *O. faveolata* from Bocas del Toro represents multiple cryptic lineages (rather than a single species), we carried out six analyses using the original data from (Dziedzic et al., 2019): 1) a Discriminant Analysis of Principal Components (DAPC), 2) a

Bayesian clustering method, 3) a neighbor joining (NJ) distance tree based on the UPGMA algorithm, 4) a species tree under the Bayesian multispecies coalescent framework of SNAPP, 5) a phylogenomic tree using the coalescent method SVDquartets, and 6) a Bayes factor contingency table to associate lineage assignment and bleaching tolerance scores.

Multi-locus raw sequence data (SRA: BioProject PRJNA413258) from 39 samples was downloaded from the National Center for Biotechnology and Information (NCBI) server. We mapped the data to a reference genome (Prada et al., 2016) and scored 383,160 Single Nucleotide Polymorphysms (SNP) following the *dDocent* pipeline (Puritz, Hollenbeck, & Gold, 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 a minimum coverage of 5x. To reduce linkage disequlibrium, 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 dataset consisted of 3,560 high-confidence SNPs. All filtering steps were done using VCFtools (Danecek et al., 2011).

Initially we examined genome-wide variation through a DAPC using the R package ADEGENET (Jombart, 2008; Jombart, Devillard, & Balloux, 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 PC axes to be retained. We then used a Bayesian clustering method as implemented in STRUCTURE 2.3.4 (Pritchard, Stephens, &

94 Donnelly, 2000) to infer the number of genetic clusters (from 1 to 8) and potential admixture.
95 Structure was performed on unlinked SNP datasets and run in parallel using StrAutoParallel v 1.0
96 (Chhatre & Emerson, 2017) using an admixture model with correlated allele frequencies. Burnin
97 was set to 250,000 followed by 500,000 MCMC generations. We evaluated the optimal *K* in
98 STRUCTURE HARVESTER (Earl, 2012) following Evanno, Regnaut, and Goudet (2005).
99 Structure results were plotted in Structure Plot (Ramasamy, Ramasamy, Bindroo, & Naik, 2014).
100
101 To further test for the presence of multiple cryptic lineages, we used a NJ tree based on
102 the UPGMA algorithm using the pairwise genetic distance matrix of genotypes with the R
103 package APE (Paradis & Schliep, 2019), with 1,000 bootstrap replicates to assess branch support.
104 We then built a species tree under the Bayesian multispecies coalescent framework of SNAPP
105 v1.3 (Bryant, Bouckaert, Felsenstein, Rosenberg, & Roy Choudhury, 2012) implemented in
106 BEAST2 v2.5 (Bouckaert et al., 2018), with a path sampling of 24 steps (MCMC length =
107 1.000,000, pre-burnin = 1,000). We excluded individuals with missing data and a dataset of
108 unlinked biallelic SNPs, no outgroup, and the genetic-clustering results used for cluster
109 assignments (Kornilios et al., 2019). Because SNAPP is computationally intensive (our analysis
110 took one month), each group included only 3 individuals, for a total of 12 individuals. Marginal
111 likelihood estimates were obtained for each different model run. Each species delimitation model
112 was ranked by their marginal likelihood estimate following Leaché, Fujita, Minin, and Bouckaert
113 (2014). Log files were combined using Log Combiner v 1.1 and input into Tracer v1.6 (Rambaut,
114 Drummond, Xie, Baele, & Suchard, 2018). Convergence and ESS > 200 were assessed using
115 Tracer after a 10% burnin. A maximum clade credibility tree was generated with Tree Annotator

v 2.3 (R. Bouckaert et al., 2018). Both the consensus tree and all tree topologies were drawn in DensiTree v2.2 (R. R. Bouckaert, 2010). Additionally, phylogenomic relationships among individuals were inferred under the coalescent method SVDquartets 1.0 (Chifman & Kubatko, 2014) implemented in PAUP* 4.0b10 (Swofford, 2003). All possible quartets were evaluated with prior assignment, using all individuals, and non-parametric bootstrapping with 1,000 replicates for branch support. iTOL was used for tree visualization and edition (Letunic & Bork, 2016).

Lastly, to test the null hypothesis of no difference in tolerance or susceptibility across cryptic lineages, we performed a contingency table Bayes factor test (Morey & Rouder, 2015) using the R package BayesFactor under an independent multinomial distribution, and estimated the difference in probability of colonies being tolerant or susceptible given their cryptic lineage.

Results

Our genetic clustering analyses based on bi-allelic and unlinked SNPs suggest the presence of three (STRUCTURE) or four (DAPC) genetic clusters (Figs. 1, 2, S1 and S2), henceforth referred to as PAN_1, PAN_2, PAN_3, and PAN_4. The NJ tree with all individual colonies recovered the same three divergent clades identified with Structure (PAN_1, PAN_3 and Pan_4), plus an extra well-supported clade within PAN_1 that corresponded to the individuals grouped as PAN_2 (Fig. 2).

The maximum clade credibility tree from SNAPP support four clades (Fig. 3A) according to the marginal likelihood estimate (Table S1). The SVDquartets tree with individuals as terminal branches was similar to the NJ tree (Fig. 2), favoring the relationships between PAN_1 with PAN_3, and PAN_2 with PAN_4, with the latter being highly supported by bootstrap iteration (Fig. 3B).

Finally, to see whether cryptic variation correlates with variation in bleaching response, we used Bayes factors. We found that bleaching susceptibility, as reported in the original study (Fig 4), and genotypic group are 42.7 times (Fig S3.A and Table S2) more likely to be associated than not associated given the observed data. For instance, the difference in probability of PAN_2 being more tolerant to bleaching than PAN_3 is greater than 0.8 (Fig S3.B). Moreover, algal symbiont genera partitioned differentially among the lineages (Fig S4). PAN_1 seems highly promiscuous associating with species from four genera: *Symbiodinium*, *Brevolium*, *Cladocopium,* and *Durusdinium*, while the other lineages are mostly restricted to *Durusdinium* species.

Discussion

Our reanalysis of Dziedzic et al.'s data suggests that *O. 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 species delimitation methods suggests that four cryptic lineages coexist under the nominal species *O. faveolata* in this Caribbean region. Furthermore, thermal tolerance occurs in three of these lineages (PAN_1, PAN_2, and PAN_4), with one of them (PAN_2) being exclusively composed of heat-tolerant

colonies, while PAN_3 composed only of heat-sensitive ones. Remarkably, symbiont affinity in these four lineages exhibits dominance of the genus *Durusdinium* in three of them (PAN_2, PAN_3, and PAN_4). In contrast, only one lineage (PAN_1) presents a promiscuous association pattern of symbiosis and associates with species from four genera.

Cryptic lineages and thermal tolerance

The prevalence of heat-tolerant colonies within the PAN_1 and_PAN_2 lineages implies that, under recurrent or stronger bleaching events, the survival of less tolerant lineages 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 grapple to capture and protect the true existing biological diversity (Richards, Berry, & van Oppen, 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 for the ensuing ecological implications this broader recognition of diversity will bring to our understating of species interactions, symbiotic relationships, physiological thresholds, and ecosystem integrity. Coral reefs' future largely lies in their physiological response

to heat, which depends directly on their symbiotic algal counterpart. Therefore, a potentially more restricted specificity among coral-algal symbioses, as it may be occurring in cryptic coral species, challenges our notions of the thermal tolerance corals can withstand under increasing warming sea surface temperatures (Thornhill, Lewis, Wham, & LaJeunesse, 2014). Although this poses an even bleaker scenario for coral reefs, it opens up an opportunity for more integrative studies that take into account cryptic diversity to define whether coral thermal tolerance is more or less widely distributed among the most common reef-builder species. Importantly, this would better inform conservation and management decisions because those species we thought of being cosmopolitan might be local or environmentally confined. Coral endangered species, such as *O. faveolata*, would be in even at greater peril as their population sizes must have been overestimated.

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

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 inherently difficulties associated with the interpretation of these markers (Fukami, 2008) but may also suggest the presence of unrecognized diversity, as we have uncovered here. It remains to be seen whether the four lineages that we delineated here occur across the Caribbean and the Gulf of Mexico, or if they occur under specific habitats at smaller geographical scales (Prada et al. 2008). Similarly, other scleractinian corals exhibit fine genetic structuring along with restricted

geographic areas that are more consistent with the existence of cryptic lineages than within-species genetic diversity (McFadden et al., 2017; Ohki, Kowalski, Kitanobo, & Morita, 2015; Richards et al., 2016; Warner et al., 2015). Therefore, 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 non-independent distribution of the genetic variation, which is constrained by the particular evolutionary history of each lineage (Sul, Martin, & Eskin, 2018). In this case, genetic variants found to be associated with thermal tolerance might have been unwarily conflated with cryptic lineages. We did not pursue a GWAS reanalysis because the small number of individuals composing each cryptic lineage would have further underpowered the inferences made from an already small sample size.

Cryptic lineages and symbiotic relationships

Highly specialized symbiotic relationships between genetically distinct hosts and their counterparts can underlie tight and long-lasting patterns of coevolution, reflecting unique prospects to withstand environmental fluctuations under particular environments, such as those projected under a climate change scenario (Voolstra et al., 2011). For hard corals, such specificity to engage in particular physiological and ecological interaction with their endosymbiotic algae translates directly into specific environmental thresholds given their dependence on symbiosis for energy acquisition (Ziegler, Eguíluz, Duarte, & Voolstra, 2018). Taxonomic revision of

symbiotic microalgae (Symbidinaceae) found in corals and other invertebrates has revealed that what it was once assumed to be a single panmictic species, it encompasses multiple genera and species with a more restricted suit of hosts (LaJeunesse et al., 2018). Whether what we considered as coral's potential for harboring diverse endosymbiotic communities might have reflected not only the taxonomic uncertainty on symbionts but also the existence of cryptic diversity among host coral species. Under this view and in light of our reanalysis, the original conclusion that *O. faveolata* could naturally harbor genetic variation to adapt to rising ocean temperatures (Dziedzic et al., 2019) could be a case in point of this richer diversity both on the coral host as well as its symbionts.

Conclusions

Accurately delineating species, even cryptic ones, is key for reef conservation and management because it allows us to accurately estimate the relative abundance and population sizes of these biological units. This, in turn, helps us understand patterns of gene flow to determine genetic connectivity among populations. Moreover, accurate detection and description of cryptic species enhance our ability to establish if ecosystem-level changes are driven by various cryptic lineages 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 current degradation experienced by coral reefs worldwide, the projected increases in

environmental variability, and regardless of the predominant 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.

References

Bálint, M., Domisch, S., Engelhardt, C. H. M., Haase, P., Lehrian, S., Sauer, J., … Nowak, C. (2011). Cryptic biodiversity loss linked to global climate change. *Nature Climate Change*, *1*(6), 313–318. doi: 10.1038/nclimate1191

Baums, I. B., Johnson, M. E., Devlin-Durante, M. K., & Miller, M. W. (2010). Host population genetic structure and zooxanthellae diversity of two reef-building coral species along the Florida Reef Tract and wider Caribbean. *Coral Reefs*, *29*(4), 835–842. doi: 10.1007/s00338-010-0645-y

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., … Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, *22*(3), 148–155. doi: 10.1016/j.tree.2006.11.004

Bouckaert, R. R. (2010). DensiTree: making sense of sets of phylogenetic trees. *Bioinformatics*, *26*(10), 1372–1373. doi: 10.1093/bioinformatics/btq110

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., … Drummond, A. J. (2018). *BEAST 2.5: An Advanced Software Platform for Bayesian Evolutionary Analysis* [Preprint]. doi: 10.1101/474296

Bryant, D., Bouckaert, R., Felsenstein, J., Rosenberg, N. A., & RoyChoudhury, A. (2012). Inferring Species Trees Directly from Biallelic Genetic Markers: Bypassing Gene Trees in a Full Coalescent Analysis. *Molecular Biology and Evolution*, *29*(8), 1917–1932. doi: 10.1093/molbev/mss086

Chhatre, V. E., & Emerson, K. J. (2017). StrAuto: automation and parallelization of STRUCTURE analysis. *BMC Bioinformatics*, *18*(1), 192. doi: 10.1186/s12859-017-1593-0

Chifman, J., & Kubatko, L. (2014). Quartet Inference from SNP Data Under the Coalescent Model. *Bioinformatics*, *30*(23), 3317–3324. doi: 10.1093/bioinformatics/btu530

Colombo-Pallotta, M. F., Rodríguez-Román, A., & Iglesias-Prieto, R. (2010). Calcification in bleached and unbleached Montastraea faveolata: evaluating the role of oxygen and glycerol. *Coral Reefs*, *29*(4), 899–907.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., … 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. doi: 10.1093/bioinformatics/btr330

DeSalvo, M. K., Sunagawa, S., Fisher, P. L., Voolstra, C. R., Iglesias-Prieto, R., & Medina, M. (2010). Coral host transcriptomic states are correlated with Symbiodinium genotypes. *Molecular Ecology*, *19*(6), 1174–1186. doi: 10.1111/j.1365-294X.2010.04534.x

Dziedzic, K. E., Elder, H., Tavalire, H., & Meyer, E. (2019). Heritable variation in bleaching responses and its functional genomic basis in reef-building corals (*Orbicella faveolata*). *Molecular Ecology*, mec.15081. doi: 10.1111/mec.15081

Earl, D. A. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361.

Edmunds, P. J., Adjeroud, M., Baskett, M. L., Baums, I. B., Budd, A. F., Carpenter, R. C., … Gates, R. D. (2014). Persistence and Change in Community Composition of Reef Corals

297 through Present, Past, and Future Climates. *PLoS ONE*, *9*(10), e107525. doi:
298 10.1371/journal.pone.0107525
299 Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals
300 using the software structure: a simulation study. *Molecular Ecology*, *14*(8), 2611–2620.
301 doi: 10.1111/j.1365-294X.2005.02553.x
302 Fišer, C., Robinson, C. T., & Malard, F. (2018). Cryptic species as a window into the paradigm
303 shift of the species concept. *Molecular Ecology*, *27*(3), 613–635. doi: 10.1111/mec.14486
304 Foll, M. (2012). BayeScan v2. 1 user manual. *Ecology*, *20*, 1450–1462.
305 Fukami, H. (2008). Short review: molecular phylogenetic analyses of reef corals. *Galaxea,*
306 *Journal of Coral Reef Studies*, *10*(2), 47–55.
307 Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Dietzel, A., Eakin, C. M., … Torda, G.
308 (2018). Global warming transforms coral reef assemblages. *Nature*, *556*(7702), 492–496.
309 doi: 10.1038/s41586-018-0041-2
310 Jombart, T. (2008). adegenet: a R package for the multivariate analysis of genetic markers.
311 *Bioinformatics*, *24*(11), 1403–1405. doi: 10.1093/bioinformatics/btn129
312 Jombart, Thibaut, Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal
313 components: a new method for the analysis of genetically structured populations. *BMC*
314 *Genetics*, *11*(1), 94.
315 Knowlton, N. (1993). Sibling species in the sea. *Annual Review of Ecology and Systematics*, 189–
316 216.
317 Kornilios, P., Thanou, E., Lymberakis, P., Ilgaz, Ç., Kumlutaş, Y., & Leaché, A. (2019).
318 Genome-wide markers untangle the green-lizard radiation in the Aegean Sea and support
319 a rare biogeographical pattern. *Journal of Biogeography*, *46*(3), 552–567. doi:
320 10.1111/jbi.13524
321 LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C.
322 R., & Santos, S. R. (2018). Systematic Revision of Symbiodiniaceae Highlights the
323 Antiquity and Diversity of Coral Endosymbionts. *Current Biology*, *28*(16), 2570-2580.e6.
324 doi: 10.1016/j.cub.2018.07.008
325 Leaché, A. D., Fujita, M. K., Minin, V. N., & Bouckaert, R. R. (2014). Species Delimitation
326 using Genome-Wide SNP Data. *Systematic Biology*, *63*(4), 534–542. doi:
327 10.1093/sysbio/syu018
328 Leaché, A. D., & Oaks, J. R. (2017). The Utility of Single Nucleotide Polymorphism (SNP) Data
329 in Phylogenetics. *Annual Review of Ecology, Evolution, and Systematics*, *48*(1), 69–84.
330 doi: 10.1146/annurev-ecolsys-110316-022645
331 Letunic, I., & Bork, P. (2016). Interactive tree of life (iTOL) v3: an online tool for the display
332 and annotation of phylogenetic and other trees. *Nucleic Acids Research*, *44*(Web Server
333 issue), W242–W245. doi: 10.1093/nar/gkw290
334 Manzello, D. P., Matz, M. V., Enochs, I. C., Valentino, L., Carlton, R. D., Kolodziej, G., …
335 Jankulak, M. (2018). Role of host genetics and heat tolerant algal symbionts in sustaining
336 populations of the endangered coral *Orbicella faveolata* in the Florida Keys with ocean
337 warming. *Global Change Biology*. doi: 10.1111/gcb.14545
338 McFadden, C. S., Haverkort-Yeh, R., Reynolds, A. M., Halàsz, A., Quattrini, A. M., Forsman, Z.
339 H., … Toonen, R. J. (2017). Species boundaries in the absence of morphological,
340 ecological or geographical differentiation in the Red Sea octocoral genus Ovabunda

(Alcyonacea: Xeniidae). *Molecular Phylogenetics and Evolution*, *112*, 174–184. doi: 10.1016/j.ympev.2017.04.025

Morey, R. D., & Rouder, J. N. (2015). *BayesFactor: Computation of Bayes factors for common designs*. *R package version 0.9. 10-1*.

NOAA, & NMFS. (2014). Endangered and Threatened Wildlife and Plants: Final Listing Determinations on Proposal To List 66 Reef-Building Coral Species and To Reclassify Elkhorn and Staghorn Corals, Final Rule, 79 FR 53851. *Federal Register Rules and Regulations*, *79*(175). Retrieved from https://www.federalregister.gov/documents/2014/09/10/2014-20814/endangered-and-threatened-wildlife-and-plants-final-listing-determinations-on-proposal-to-list-66

Ohki, S., Kowalski, R. K., Kitanobo, S., & Morita, M. (2015). Changes in spawning time led to the speciation of the broadcast spawning corals Acropora digitifera and the cryptic species Acropora sp. 1 with similar gamete recognition systems. *Coral Reefs*, *34*(4), 1189–1198. doi: 10.1007/s00338-015-1337-4

Paradis, E., & Schliep, K. (2019). ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*(3), 526–528.

Prada, C., & Hellberg, M. E. (2013). Long prereproductive selection and divergence by depth in a Caribbean candelabrum coral. *Proceedings of the National Academy of Sciences*, *110*(10), 3961–3966. doi: 10.1073/pnas.1208931110

Prada, Carlos, Hanna, B., Budd, A. F., Woodley, C. M., Schmutz, J., Grimwood, J., … Medina, M. (2016). Empty Niches after Extinctions Increase Population Sizes of Modern Corals. *Current Biology*, *26*(23), 3190–3194. doi: 10.1016/j.cub.2016.09.039

Prada, Carlos, McIlroy, S. E., Beltrán, D. M., Valint, D. J., Ford, S. A., Hellberg, M. E., & Coffroth, M. A. (2014). Cryptic diversity hides host and habitat specialization in a gorgonian-algal symbiosis. *Molecular Ecology*, *23*(13), 3330–3340. doi: 10.1111/mec.12808

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959.

Puritz, J. B., Hollenbeck, C. M., & Gold, J. R. (2014). *dDocent* : a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. *PeerJ*, *2*, e431. doi: 10.7717/peerj.431

Ramasamy, R. K., Ramasamy, S., Bindroo, B. B., & Naik, V. G. (2014). STRUCTURE PLOT: a program for drawing elegant STRUCTURE bar plots in user friendly interface. *SpringerPlus*, *3*(1), 431.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). *Posterior summarisation in Bayesian phylogenetics using Tracer 1.7*. 3.

Richards, Z. T., Berry, O., & van Oppen, M. J. H. (2016). Cryptic genetic divergence within threatened species of Acropora coral from the Indian and Pacific Oceans. *Conservation Genetics*, *17*(3), 577–591. doi: 10.1007/s10592-015-0807-0

Rippe, J. P., Matz, M. V., Green, E. A., Medina, M., Khawaja, N. Z., Pongwarin, T., … Davies, S. W. (2017). Population structure and connectivity of the mountainous star coral, *Orbicella faveolata* , throughout the wider Caribbean region. *Ecology and Evolution*. doi: 10.1002/ece3.3448

Rosser, N. L. (2015). Asynchronous spawning in sympatric populations of a hard coral reveals cryptic species and ancient genetic lineages. *Molecular Ecology*, *24*(19), 5006–5019. doi: 10.1111/mec.13372

Severance, E. G., & Karl, S. A. (2006). Contrasting population genetic structures of sympatric, mass-spawning Caribbean corals. *Marine Biology*, *150*(1), 57–68. doi: 10.1007/s00227-006-0332-2

Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C., … Moore, P. J. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change*, *9*(4), 306–312. doi: 10.1038/s41558-019-0412-1

Struck, T. H., Feder, J. L., Bendiksby, M., Birkeland, S., Cerca, J., Gusarov, V. I., … Dimitrov, D. (2018). Finding Evolutionary Processes Hidden in Cryptic Species. *Trends in Ecology & Evolution*, *33*(3), 153–163. doi: 10.1016/j.tree.2017.11.007

Sul, J. H., Martin, L. S., & Eskin, E. (2018). Population structure in genetic studies: Confounding factors and mixed models. *PLOS Genetics*, *14*(12), e1007309. doi: 10.1371/journal.pgen.1007309

Swofford, D. L. (2003). PAUP*: Phylogenetic analysis using parsimony version 4.0 b10. *Sinauer Associates, Sunderland*.

Thornhill, D. J., Lewis, A. M., Wham, D. C., & LaJeunesse, T. C. (2014). Host-specialist lineages dominate the adaptive radiation of reef coral endosymbionts: adaptive radiation of symbiotic dinoflagellates. *Evolution*, *68*(2), 352–367. doi: 10.1111/evo.12270

van Woesik, R., Sakai, K., Ganase, A., & Loya, Y. (2011). Revisiting the winners and the losers a decade after coral bleaching. *Marine Ecology Progress Series*, *434*, 67–76. doi: 10.3354/meps09203

Voolstra, C. R., Sunagawa, S., Matz, M. V., Bayer, T., Aranda, M., Buschiazzo, E., … Medina, M. (2011). Rapid Evolution of Coral Proteins Responsible for Interaction with the Environment. *PLoS ONE*, *6*(5), e20392. doi: 10.1371/journal.pone.0020392

Warner, P. A., van Oppen, M. J. H., & Willis, B. L. (2015). Unexpected cryptic species diversity in the widespread coral *Seriatopora hystrix* masks spatial-genetic patterns of connectivity. *Molecular Ecology*, *24*(12), 2993–3008. doi: 10.1111/mec.13225

Wham, D. C., & LaJeunesse, T. C. (2016). Symbiodinium population genetics: testing for species boundaries and analysing samples with mixed genotypes. *Molecular Ecology*, *25*(12), 2699–2712. doi: 10.1111/mec.13623

Wright, R. M., Correa, A. M. S., Quigley, L. A., & Davies, S. W. (2019). *Gene expression of endangered coral (* Orbicella *spp.) in the Flower Garden Banks National Marine Sanctuary after Hurricane Harvey* [Preprint]. doi: 10.1101/703447

Ziegler, M., Eguíluz, V. M., Duarte, C. M., & Voolstra, C. R. (2018). Rare symbionts may contribute to the resilience of coral–algal assemblages. *The ISME Journal*, *12*(1), 161–172. doi: 10.1038/ismej.2017.151

Data Accessibility

- Sequence data archived at NCBI's Sequence Read Archive (SRA) BioProject PRJNA413258.

- Scripts used for analysis will be made publicly available at https://github.com/matiasgoco

Author Contributions

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

Tables and Figures

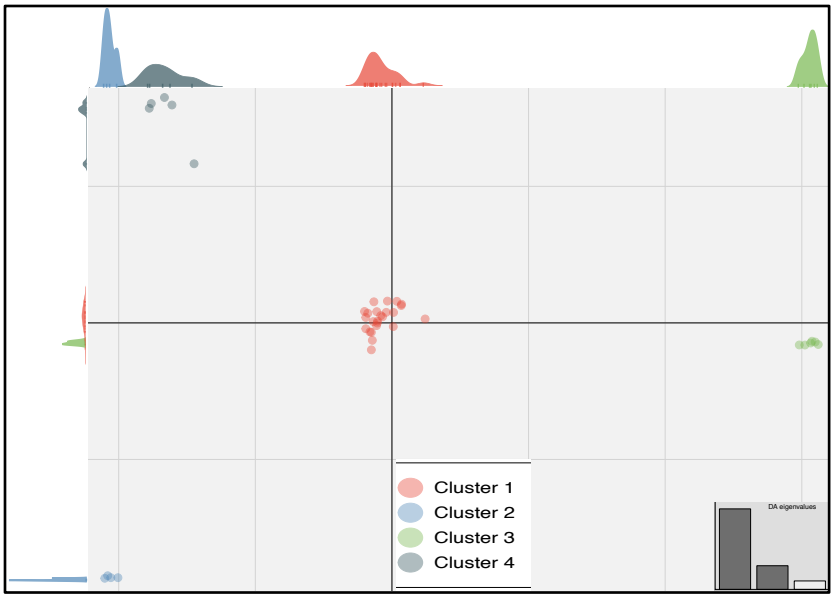

Figure 1. Discriminant analysis of principal components (DAPC) (Optimal $k = 4$ and number of PCA = 6). Note the full segregation of the data, suggestive of non-randomly reproducing groups.

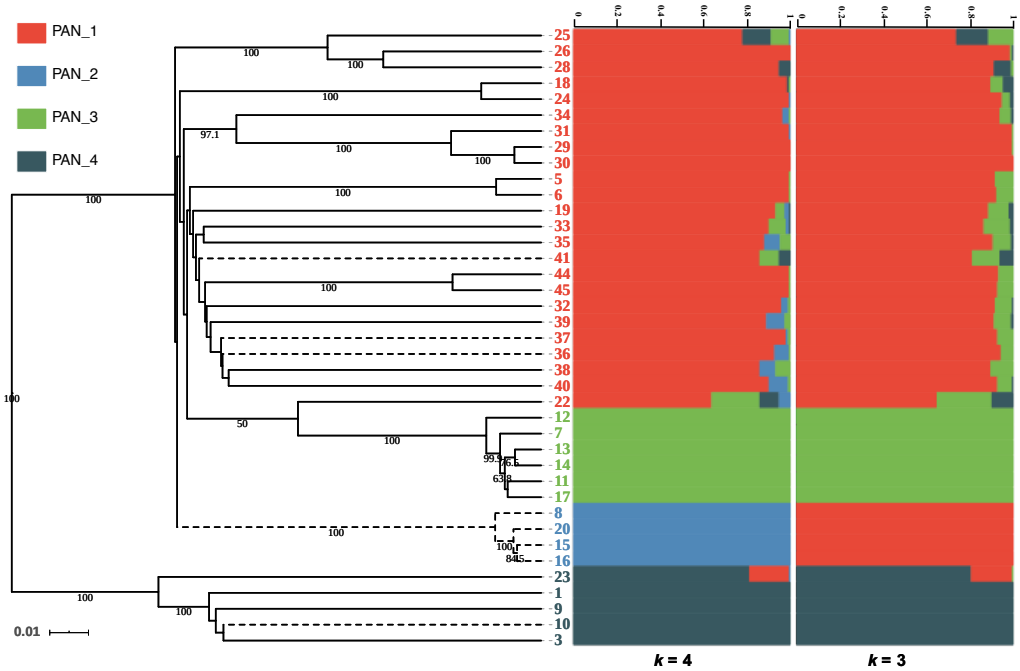

Figure 2. Neighbor Joining (NJ) tree using the pairwise genetic distance matrix (node numbers are bootstrap support values), depicting the groups inferred from the genetic clustering methods (dashed branches represent heat-tolerant samples). Hierarchical Bayesian population clustering with STRUCTURE depicting $k = 3$ (optimal) and $k = 4$.

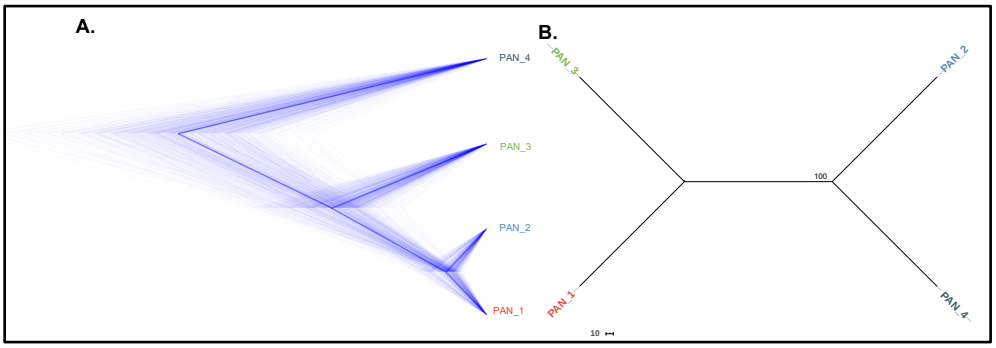

Figure 3. A) Trees inferred from the Bayesian coalescence analysis of SNAPP (the congruent tree is in thick blue), B) SVDquartets tree from analysis in PAUP*.

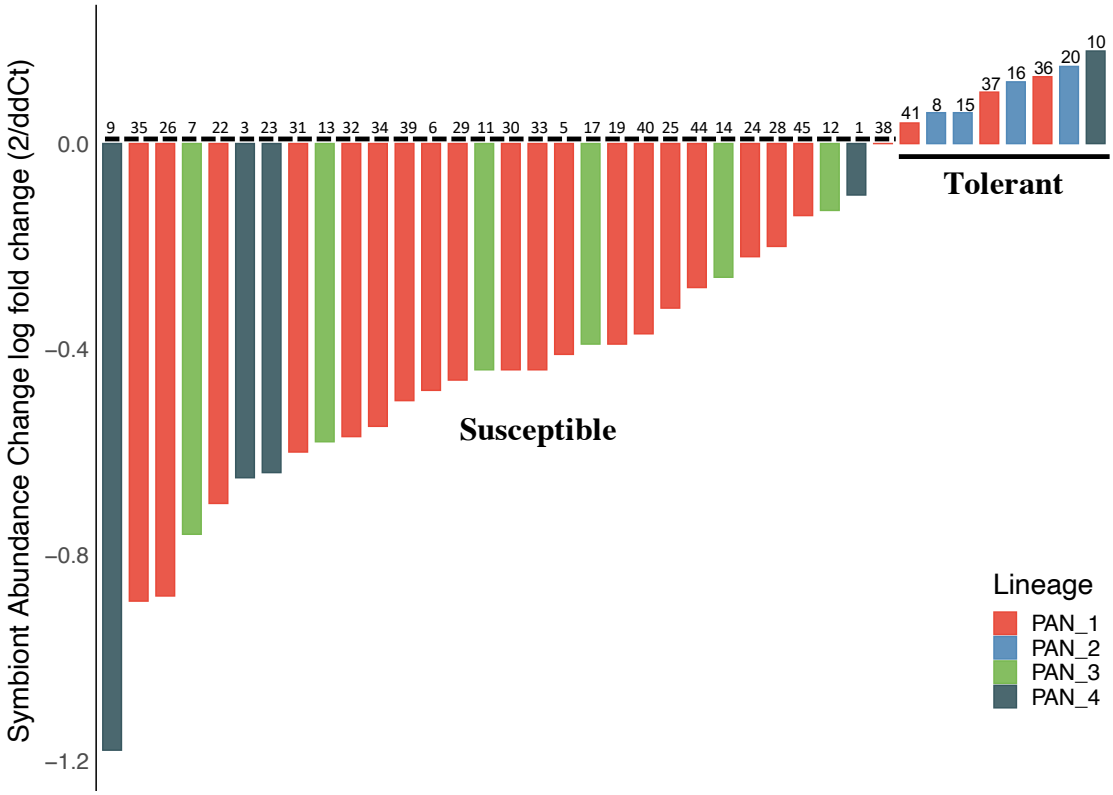

Figure 4. Log-fold change (2^{-ddCt}) algal symbiont abundance per colony using qPCR after four weeks of experimental conditions (Modified from Dziedzic et al., 2019). Colors denote the cryptic lineages uncovered in this study.

Supplementary material

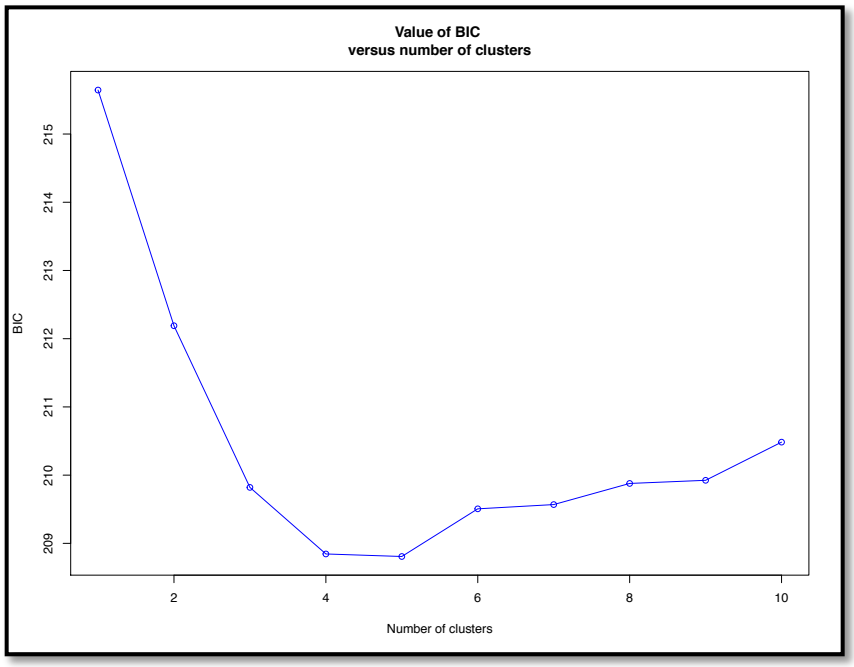

Figure S1. Value of Bayes information criterion versus number of clusters found in DAPC.

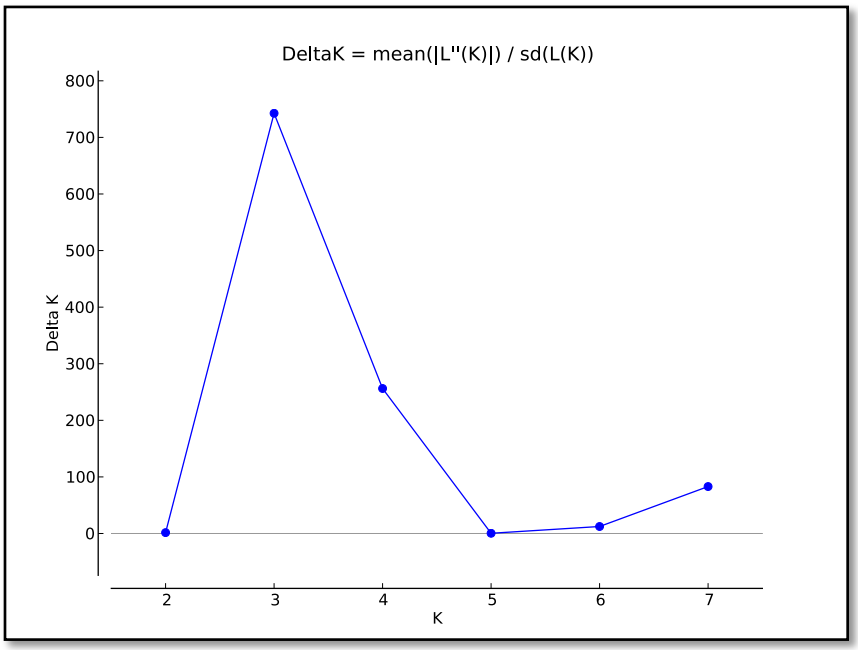

Figure S2. Most probable number of clusters according to the value of Delta K in Structure.

No. Groups	Marginal Likelihood Estimate	Rank
2	7292164.4623	3
3	11303237.4275	2
4	14163377.8363	1

Table S1. Path sampling results for the four clusters delimitation models.

Bleaching	Genotypic group			
	PAN_1	PAN_2	PAN_3	PAN_4
Tolerant	3	4	0	1
Susceptible	20	0	6	4

Table S2. Frequencies for tolerant and susceptible colonies to bleaching according to their genotype group

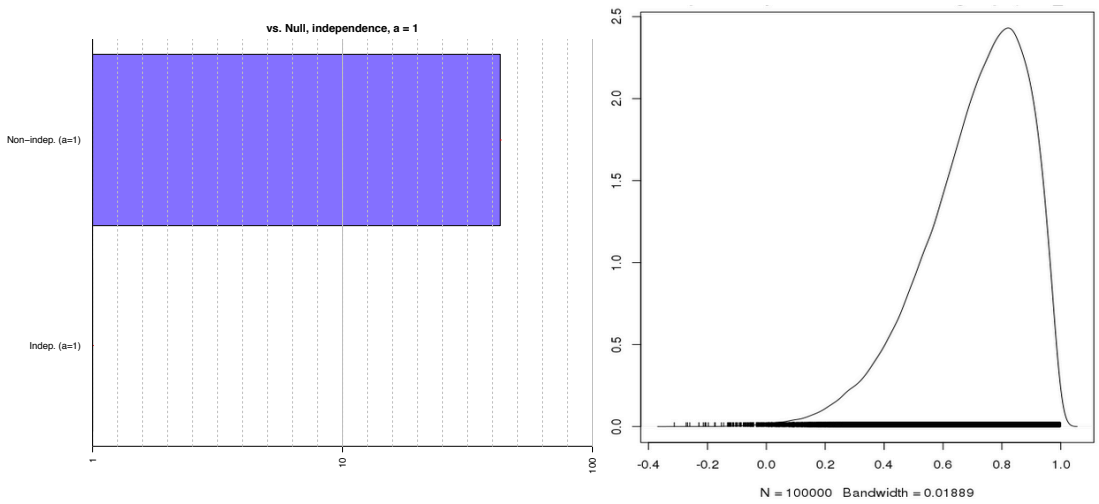

Figure S3. A) Bayes factor plot of contrasting hypothesis of non-association (H0) vs association (H1) between genotypic group and bleaching tolerance. **B)** Increase in probability of bleaching tolerance of genotypic group PAN_2 when compared to PAN_3.

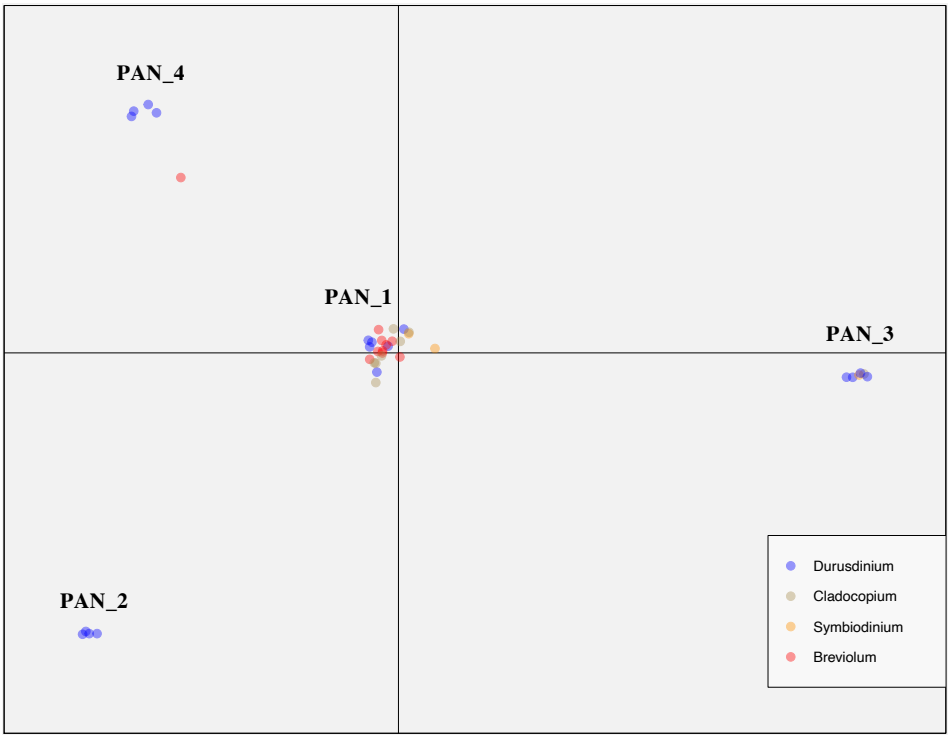

Figure S4. Discriminant analysis of principal components (DAPC) as in Fig.1 but colored by symbiont genera, previously known as clades.