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Genomic variation of an endosymbiotic dinoflagellate (*Symbiodinium 'fitti'*) among closely related coral hosts

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Abstract

Mutualisms where hosts are coupled metabolically to their symbionts often exhibit high partner fidelity. Most reef-building coral species form obligate symbioses with a specific species of photosymbionts, dinoflagellates in the family Symbiodiniaceae, despite needing to acquire symbionts early in their development from environmental sources. Three Caribbean acroporids (*Acropora palmata*, *A. cervicornis* and their F_1 hybrid) are sympatric across much of their range, but often occupy different depth and light habitats. Throughout this range, both species and their hybrid associate with the endosymbiotic dinoflagellate *Symbiodinium* '*fitti*'. Because light (and therefore depth) influences the physiology of dinoflagellates, we investigated whether *S.* '*fitti*' populations from each host taxon were differentiated genetically. Single nucleotide polymorphisms (SNPs) among *S.* '*fitti*' strains were identified by aligning shallow metagenomic sequences of acroporid colonies sampled from across the Caribbean to a ~600-Mb draft assembly of the *S.* '*fitti*' genome (from the CFL14120 *A. cervicornis* metagenome). Phylogenomic and multivariate analyses revealed that genomic variation among *S.* '*fitti*' strains partitioned to each host taxon rather than by biogeographical origin. This is particularly noteworthy because the hybrid has a sparse fossil record and may be of relatively recent origin. A subset (37.6%) of the SNPs putatively under selection were nonsynonymous mutations predicted to alter protein efficiency. Differences in genomic variation of *S.* '*fitti*' strains from each host taxon may reflect the unique selection pressures created by the microenvironments associated with each host. The nonrandom sorting among *S.* '*fitti*' strains to different hosts could be the basis for lineage diversification via disruptive selection, leading to ecological specialization and ultimately speciation.

KEYWORDS
Acropora, hybrid, niche diversification, single-nucleotide polymorphisms, Symbiodiniaceae, symbiosis

1 | INTRODUCTION

Ecosystem services provided by coral–dinoflagellate mutualisms rival the contributions of other widely studied symbioses. In the coral–dinoflagellate mutualism, each partner benefits as the coral receives photosynthetic sugars from their dinoflagellate symbionts and the algal symbiont receives nutrients and protection in return (Trench, 1979). Though decades of investigations have probed the

causes and consequences of coral–algal dysbiosis (coral bleaching), we are still gathering information on their basic biology (Cziesielski et al., 2019). Specifically, little is known about the intraspecies level of co-evolutionary dynamics between hosts and their symbionts. The interdependence of the partners adds complexity to the system as each partner is selected in the context of the other.

Pinpointing the mechanisms driving the widespread occurrences of host–symbiont specificity, or lack thereof, between coral species and their Symbiodiniaceae species is important for understanding the basic evolutionary biology of coral–dinoflagellate mutualisms. Corals have co-evolved with the Symbiodiniaceae since the Jurassic period (LaJeunesse et al., 2018) and, over time, have become uniquely adapted to their dinoflagellate symbionts (Forsman et al., 2020; LaJeunesse et al., 2004). While corals in early life stages often host several symbiont species, this community wanes over time to the dominant symbiont species (Abrego et al., 2009; Coffroth et al., 2001; Poland & Coffroth, 2017). This suggests that coral species are most compatible with their dominant symbiont species and foreign species pairings might be maladaptive (Pettay et al., 2015). Improved understanding of fine-scale host and symbiont population dynamics is desirable to better recognize the complexities of partner compatibility. Symbiont and host pairings that span multiple taxa (i.e., a symbiont species that can associate with multiple host species corals or vice versa) are of particular interest for revealing the physiological and ecological ramifications of partner pairings.

The mutualism between Caribbean acroporids (*Acropora* spp.) and the endosymbiotic dinoflagellate *Symbiodinium* '*fitti*' [nomen nudum senso Pinzón et al. (2011)] is ideal for studying partner population dynamics over a large biogeographical range. These coral taxa are found throughout the Caribbean and often differentiate across a depth and light gradient; *Acropora cervicornis* occupies a lower light habitat (~10 m depth) relative to its high-light-dwelling (~3 m depth) sibling species *Acropora palmata* and their hybrid (~1 m depth; Fogarty, 2012; Goreau, 1959; LaJeunesse, 2002). Morphology ranges from broad, moose antler branches in *A. palmata* to thinner, stag antler branches in *A. cervicornis* with resulting differences in the flow and light field within and around the colonies (Enríquez et al., 2017; Gladfelter, 1983, 2007). Morphological differences thus may result in unique specialization requirements for the light-dependent symbionts associated with each host taxon. All three coral taxa harbour *S.* '*fitti*' (ITS2 type A3), which is distinct from *Symbiodinium* A3 lineages found in giant clams and other cnidarians (Kemp et al., 2015; Lee et al., 2015; Pinzón et al., 2015; Shoguchi et al., 2018). Though adult colonies of Caribbean acroporids primarily associate with *S.* '*fitti*', they can occasionally harbour other genera of Symbiodiniaceae (*Breviolum* spp., *Durusdinium trenchii* and *Cladocopium* spp.), but these associations are often transitory and revert to *S.* '*fitti*' over time (Baums et al., 2010; Thornhill et al., 2006). The association of *S.* '*fitti*' with three host taxa at a range of depths across a large geographical region provides a unique opportunity to study how abiotic selective processes may influence symbiont adaptation and co-evolution with host(s).

The ecological and evolutionary dynamics between host and symbiont species are influenced by differences in their reproduction and dispersal strategies (reviewed in Thornhill et al., 2017). Caribbean acroporid corals reproduce via production of planktonic larvae and also disperse locally via fragmentation. Gene flow is restricted between the eastern and western Caribbean regions for *A. palmata* and *A. cervicornis* (Baums et al., 2005, 2006). Within each region, further population structure is observed but the specifics differ between species, with *A. cervicornis* showing generally more fine-scale differentiation than *A. palmata* (Baums et al., 2005, 2006; Devlin-Durante & Baums, 2017; Drury et al., 2016; Hemond & Vollmer, 2010; Vollmer & Palumbi, 2002, 2006). *A. palmata* and *A. cervicornis* have been present in the fossil record since the late Pliocene (~2.6–3.6 million years ago) whereas the hybrid is mostly absent from the fossil record (Budd & Johnson, 1999; McNeill et al., 1997; Precht et al., 2019). Regardless of the differences in evolutionary history and population structure among the Caribbean acroporids, their ecological success is tied to the presence of *S.* '*fitti*'.

The population structure and genotypic diversity of *S.* '*fitti*' has received less attention and at a coarser level of genomic resolution (Baums et al., 2014, 2019; Thornhill et al., 2017). When compared to one of its hosts (*A. palmata*), *S.* '*fitti*' has more population genetic structure and thus perhaps more limited dispersal capability (Baums et al., 2014; Fitt & Trench, 1983; Thornhill et al., 2017). Though *S.* '*fitti*' cells divide mitotically within the host, sexual reproduction in Symbiodiniaceae has not been ruled out as a reproductive strategy because recombination is evident in population genetic data (Baums et al., 2014) and meiotic machinery has been detected in genomic data (Bellantuono et al., 2019; Chi et al., 2014; Levin et al., 2016; Shah et al., 2020). The stark differences between the life-history strategies of the two partners may contribute to the higher levels of population structure of *S.* '*fitti*' compared to *A. palmata* throughout the Caribbean (Baums et al., 2014; Thornhill et al., 2017). Expanding the evaluation of *S.* '*fitti*' population structure to include associations with all three acroporid hosts can place host–symbiont population dynamics in a broader evolutionary context.

Here, we describe fine-scale genetic differences in *S.* '*fitti*' strains across its three host taxa spanning the geographical distribution of the mutualism. A draft *S.* '*fitti*' genome assembly was constructed from *A. cervicornis* metagenomic sequences and compared to other Symbiodiniaceae genomic resources. Variation in genome-wide single nucleotide polymorphisms (SNPs) was investigated in *S.* '*fitti*' and scanned for mutations that may change protein structure and function. Lastly, the potential biological and evolutionary ramifications of the genomic variation of *S.* '*fitti*' are discussed.

2 | METHODS

2.1 | Sample collection, sequencing and assembly

Tissue was collected for genome sequencing from 76 acroporids spanning the geographical distribution of *Symbiodinium* '*fitti*' (Figure 1; Table S1, Kitchen et al., 2019). All samples were collected from the tips of adult colonies. High-molecular-weight DNA was

isolated from each coral tissue sample using the Qiagen DNeasy kit (Qiagen) without prior enrichment for *S.* '*fitti*'. Of these samples, one specimen for each species from Florida (*Acropora cervicornis* CFL14120 and *Acropora palmata* PFL1012) was "deeply" sequenced (~150× coverage; Kitchen et al., 2019). Paired-end short insert (550 nt) sequencing libraries of the two deeply sequenced samples were constructed with 1.8–2 µg sample DNA and the TruSeq DNA PCR-Free kit (Illumina). The remaining 74 paired-end short insert (350 nt) sequencing libraries were constructed using 100 ng sample DNA and the TruSeq DNA Nano kit (Illumina) with coverage of 8–40× (Kitchen et al., 2019). Deep- and shallow-sequence libraries were pooled separately and sequenced on either Illumina HiSeq 2500 or HiSeq 4000 (Table S1; Illumina).

Sequencing adaptors and low-quality base calls (Phred score <25) from the 3′ end of the deeply sequenced *A. cervicornis* metagenome reads were trimmed using CUTADAPT version 1.6 (Martin, 2011). After initial filtering, processed reads shorter than 50 bp were discarded and PCR (polymerase chain reaction) duplicates removed using FASTUNIQ version 1.1 (Xu et al., 2012). A series of filtering steps were completed to identify the fraction of reads originating from *A. cervicornis* and Symbiodiniaceae. First, a modified approach similar to BLOBOLOGY, which compares sequence similarity, read coverage and GC content, was performed (Kumar et al., 2013). Contigs from a preliminary genome assembly created with SOAPDENOVO2 version 0.4 (parameters -K 95 –R) were compared for similarity against the coral *Acropora digitifera* (NCBI: GCF_000222465.1) and symbiont *Breviolum minutum* (OIST: symbB.v1.0.genome.fa) genomes, and NCBI nucleotide database (nt) using MEGABLAST (*e* value 1e-5; Altschul et al., 1997; Shinzato et al., 2011; Shoguchi et al., 2013). Contigs identified as symbiont or as other contaminants generally had lower sequence coverage than the contigs more similar to *A. digitifera*, but no clear peak in GC content was observed in the symbiont fraction (GC content ranges from 21% to 65%, data not shown). Contigs that had matched to the nt database but not to either coral or symbiont genome were used to create a local contamination database to further screen the reads (Luo et al., 2015).

Trimmed reads were aligned with BOWTIE2 version 2.2.9 (parameters –q –fast; Langmead & Salzberg, 2012) consecutively to the *A. digitifera* mitochondria (NBCI: KF448535.1), followed by a concatenated set of three Symbiodiniaceae genomes (*Symbiodinium microadriaticum*, *Breviolum minutum*, *Fugacium kawagutii*; Aranda et al., 2016; Lin et al., 2015; Shoguchi et al., 2013), and the contamination database. This filtering step, however, only aligned 0.28% of trimmed reads from *A. cervicornis* to the Symbiodiniaceae genomes. Reads that mapped to Symbiodiniaceae genomes (*n* = 1,004,992) were extracted and assembled using SPADES version 3.9.1 with a multi-kmer approach (-k 21,33,55,77,99; Bankevich et al., 2012). The reads that aligned to the contamination database were assembled separately

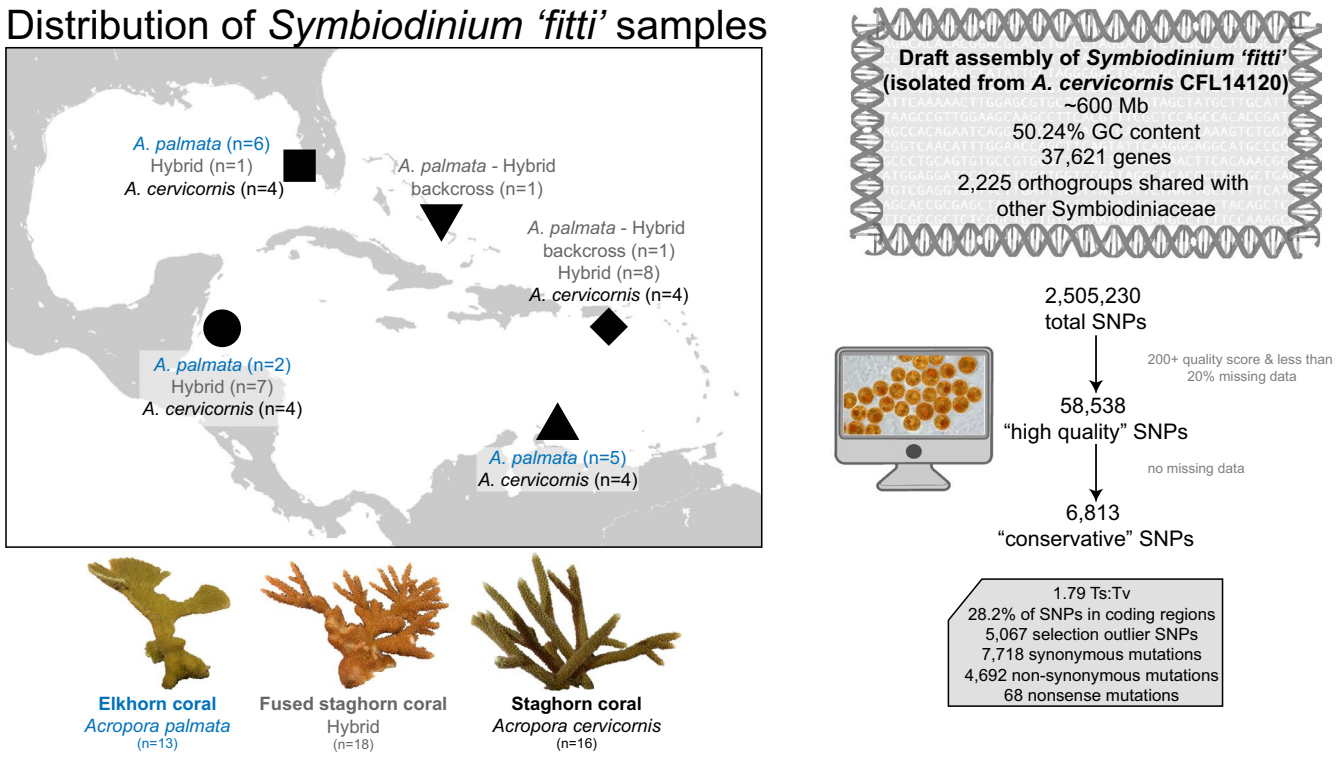

FIGURE 1 Sampling design and summary statistics of the population genomic approach used to characterize *Symbiodinium* '*fitti*' across three coral hosts. *Acropora palmata* (*n* = 13), *Acropora cervicornis* (*n* = 16) and their F_1 hybrid (*n* = 18) samples used for shallow genome sequencing spanned their geographical distribution (left). Summary statistics for the "deeply sequenced" CFL 14120 *A. cervicornis*–*S.* '*fitti*' draft genome assembly including overall length, %GC content (50.24%), number of genes (37,621) and shared gene families with other Symbiodiniaceae (2225; right). Given are summary statistics and visual depiction of the quality filtering work flow that was employed to identify high-quality variants and those that are under selection (right). Coral images from N. Fogarty and I. Baums [Colour figure can be viewed at wileyonlinelibrary.com]

with SPADES version 3.9.1 as described above, resulting in three additional contigs that matched Symbiodiniaceae genomes through BLAST similarity.

The filtered *A. cervicornis* reads were assembled with SOAPDENOVO version 2, followed by six rounds of gap filling using GAPCLOSER version 1.12 and scaffolding using both SSPACE version 2.0 (rounds 1, 3 and 5) and LINKS version 1.8.5 with *A. digitifera* scaffolds as the "long-reads" (-t 2 –d 3000 –k 25, rounds 2, 4 and 6) on alternate rounds (Boetzer & Pirovano, 2014; Luo et al., 2015; Warren et al., 2015). After the first three rounds and then each subsequent round, the contigs/scaffolds were partitioned to either coral or symbiont based on the top scoring match (lowest e-value) against a local blast database containing three Symbiodiniaceae genomes (*Symbiodinium microadriaticum*, *Breviolum minutum*, *Fugacium kawagutii*) and five cnidarian genomes (*Hydra*, *Hydractinia*, *Nematostella*, *Exaiptasia*, *Acropora digitifera*). If the scaffolds equally matched cnidarian and symbiont sequences or did not match either they were retained in the coral fraction. Scaffolds identified as Symbiodiniaceae after the six rounds as well as those assembled with SPADES above were combined. Two additional rounds of scaffolding with LINKS using the *S. microadriaticum* genome assembly as "long-reads" followed by SSPACE and gap filling with GAPCLOSER were performed. To remove any remaining sequences matching cnidarian sequences, a final round of scaffold partitioning was performed by comparing the scaffolds against the *Acropora* spp. genomes: *A. digitifera* (Shinzato et al., 2011), *A. palmata* (S.A. Kitchen et al., unpublished; http://baumslab.org/research/data), *A. cervicornis* (S.A. Kitchen et al., unpublished), *A. hyacinthus* (Voolstra et al., 2015) and *A. tenuis* (Liew et al., 2016); and *Symbiodinium* spp. genomes: *S. microadriaticum* (Aranda et al., 2016) and *S. tridacnidorum* (Shoguchi et al., 2018).

2.2 | Repeat masking, genome annotation and completeness

Repetitive sequences were predicted with REPEATMODELER version 1.0.11 (Smit & Hubley 2008–2015), filtered for genuine genes based on BLAST similarity to the NCBI nr database (e-value 1e-5), and masked using REPEATMASKER version 4.0.7 (Smit et al., 1996–2010). Genes were predicted using *ab initio* tools AUGUSTUS version 3.2.3 (Stanke et al., 2006) trained with the conserved genes predicted from BUSCO ALVEOLATA version 10 (Simão et al., 2015) and GENEMARK-ES version 4.38 (Lomsadze et al., 2005) using modified parameters previously reported (Chen et al., 2020), and protein alignments using the tool GEMOMA (Keilwagen et al., 2019) with updated gene models predicted from six Symbiodiniaceae genomes (Chen et al., 2020). Gene predictions were combined using EVIDENCEMODELER (Haas et al., 2008) according to modifications previously reported (Chen et al., 2020) with weights of 1 for both *ab initio* and protein alignment predictions.

Each predicted gene in *S.* '*fitti*' was queried against the NCBI nr, Uniprot SwissProt and trembl databases using BLASTX 2.6.0+ (max target seqs =5, max hsps =1, *e*-value =1*e*-5; Altschul et al., 1997; Apweiler et al., 2004; Bairoch & Apweiler, 1997; UniProt, 2014). Gene models were also compared to the *S. microadriaticum* gene and protein predictions (NCBI: GCA_001939145.1) using BLASTP (Altschul et al., 1997). Assembly statistics, genome completeness and gene metrics of the *S.* '*fitti*' assembly were compared to *S. microadriaticum* (Aranda et al., 2016) and *S. tridacnidorum* (Shoguchi et al., 2018) using an online version of CEGMA with the eukaryote orthologue set executed by GVOLANTE (https://gvolante.riken.jp) and gene metric statistics from Chen et al., (2020). ORTHOFINDER version 2.4.0 with default settings (Emms & Kelly, 2015) was used to identify unique and shared orthogroups between *S.* '*fitti*' and 11 other Symbiodiniaceae species used above for gene prediction.

2.3 | *S.* '*fitti*' infection status

The presence of multiple *S.* '*fitti*' strains within a coral host sample was determined using 12 *S.* '*fitti*'-specific microsatellite loci as described by Baums et al. (2014). *S.* '*fitti*' is haploid, and thus samples with multiple alleles for any given *S.* '*fitti*' microsatellite locus were deemed co-infected and removed from downstream analyses (Table S1).

2.4 | Variant detection and filtering

we will want to multi-thread this step (it is good, but SLOW); also beef up memory

For SNP analyses, the *S.* '*fitti*' genome assembly based on the *A. cervicornis* metagenome was used as a reference for variant calling of the deeply sequenced *A. palmata* and all shallow genome samples (Table S1). The 47 shallow and one deep sequenced genome samples were aligned using BWA version 0.7.15 (Li, 2013). SAMTOOLS version 1.4 was used to remove PCR duplicates from the BAM file and alignment statistics were calculated using SAMTOOLS *flagstat* (Table S1; Li et al., 2009). Variants were gathered using SAMTOOLS *mpileup* using the –ugAEf and –t AD,DP flags and called using BCFTOOLS version 1.4 using the haploid, –f GQ and -vmO z flags (Li et al., 2009; Narasimhan et al., 2016). The BCFTOOLS (Li et al., 2009; Narasimhan et al., 2016) –m2 –M2 –v snps flags were used to separate SNPs from the output and the –v indels flag was used to remove indels from the output (Narasimhan et al., 2016). "High-quality" SNPs and indels were characterized as variants with a quality score over 200 and with no more than 20% of variant calls missing at a given site among all samples (Danecek et al., 2011; Narasimhan et al., 2016). The remaining 58,538 "high-quality" SNPs were used in subsequent analyses.

2.5 | Population structure

The *psbA* minicircle was assembled from each sample to determine if the dominant algal partners among the three host taxa were all *S.* '*fitti*'. The *psbA* minicircle in the *S.* '*fitti*' genome assembly was identified through BLAST searches against three *psbA* sequences from NCBI (JN557866.1 = *Symbiodinium* type A3, JX094319.1 = *Breviolum minutum*, and AJ884898.1 = *B. faviinorum*; Barbrook et al., 2006; Mungpakdee et al., 2014; Pochon et al., 2012). The *psbA* minicircle for

the remaining samples was assembled using two approaches. In the first, filtered and trimmed short-read sequences were mapped to *S.* '*fitti*' *psbA* sequence (scaffold71443) using BOWTIE 2 version 2.3.4.1 (Langmead & Salzberg, 2012) with the --sensitive mode parameter. Mapped reads were extracted using BEDTOOLS version 2.26.0 (Quinlan & Hall, 2010) and assembled using SPADES version 3.10.1 (Bankevich et al., 2012) with various k-mer sizes (-k 21, 33, 55, 77 and 99). In the second approach, the *de novo* organelle genome assembler NOVOPLASTY was used (Dierckxsens et al., 2016). The *S.* '*fitti*' genome *psbA* sequence was used as the seed sequence to extract similar sequences from the original, unfiltered reads for each sample. A consensus sequence from the two approaches for each sample was created after manual alignment of the sequences using MEGA6 (Tamura et al., 2013).

Phylogenomic patterns of *S.* '*fitti*' genomic variation were determined using the 6,813 high-quality SNPs without missing data (hereafter referred to as "conservative" SNPs) with the RAXML-NG version 0.9.0 GTR+FO+G nucleotide model (Stamatakis, 2014). The tree topology with the highest likelihood score is presented with nodal support from 100 bootstrap replicates (Stamatakis, 2014). Population structure was evaluated using STRUCTURE version 2.3.4 using haploid, default settings for the 58,813 "high-quality" SNPs (Pritchard et al., 2000). Additionally, the R package POPPR version 2.1.0 was used to determine the multilocus genotype of each strain using "high-quality" SNPs with different genetic distance thresholds ranging from 10% to 20% (Table S1; Kamvar et al., 2014; Kitchen et al., 2020). Clusters in multivariate space were detected using the *pca* function in PCADAPT for the "high-quality", selection outlier and gene region SNPs (Luu et al., 2017). An analysis of molecular variance (AMOVA, POPPR R package) was used for additional detection of population differentiation (Kamvar et al., 2014). One AMOVA was stratified (population/subpopulation) by coral taxa/geographical location and the second by geographical location/coral taxa.

2.6 | Determination of variants under selection

Two different methods were used to identify candidate loci under selection. BAYESCAN version 2.1 is a Bayesian method that incorporates uncertainty of allele frequencies between populations with small sample sizes (Fischer et al., 2011; Foll et al., 2010; Foll & Gaggiotti, 2008). The default BAYESCAN settings were used for determining SNPs under selection when accounting for *S.* '*fitti*' host, location, and host × location interactions. PCADAPT version 4.0.3 was used to determine SNPs under selection without prior population information using the default settings (Knaus & Grünwald, 2017). Outliers from BAYESCAN were determined as markers where false discovery rate (FDR) <0.05 and outliers from PCADAPT version 4.0.3 were determined by using the QVALUE package to calculate *q* value <0.05 (alpha; Fischer et al., 2011; Foll et al., 2010; Foll & Gaggiotti, 2008; Knaus & Grünwald, 2017; Storey et al. 2020). All statistics from SNPs under selection, their proximity to coding regions, sequence coverage and per SNP F_{ST} are given in Tables S7 and S9. SNPEFF version 4.3 was used to predict downstream functional implications of all detected variants (De Baets et al., 2011).

2.7 | Data and code availability

Raw data are publicly available on NCBI under SRA project PRJNA473816. Code for data analysis and figure generation are available on github (https://github.com/hgreich/Sfitti). Draft assemblies for the reference *A. cervicornis* – *S.* '*fitti*' (CFL14120) are on dryad (https://doi.org/10.5061/dryad.xgxd254g8).

3 | RESULTS

3.1 | Genome statistics and comparison to other Symbiodiniaceae

The *Symbiodinium* '*fitti*' assembly has a total nucleotide length of over 600 Mb (601,782,011 bp), of which 17.86% is predicted to be repetitive sequences and contains 274,185 contigs/scaffolds (*Acropora cervicornis*–*S.* '*fitti*' CFL14120; Figure 1; Table S2). The *Acropora palmata*–*S.* '*fitti*' (PFL1012) deeply sequenced sample had 297,371,995 reads map to the *A. cervicornis*–*S.* '*fitti*' reference (19% mapping rate, 8.5% paired reads, 4.2% singleton reads; Table S1). The shallow-sequenced genome samples with one *S.* '*fitti*' strain had an average of 6,461,332 reads map to the reference (19.2% mapping rate, 8.8% paired reads, 4.3% singleton reads; Table S1). The GC content and number of ambiguous bases were comparable to other *Symbiodinium* spp. assemblies at 50.24% and 4.82%, respectively (Figure 1; Table S2; González-Pech et al., 2021). The genome completeness was assessed by the identification of the 248 core genes queried using the CEGMA program. The *S.* '*fitti*' assembly had 55 complete proteins, 79 complete + partial proteins and 169 missing proteins, which was lower than other *Symbiodinium* spp. queried (Table S2). The average number of orthologues per core gene was ~1.45, which was comparable to the other *Symbiodinium* spp. assemblies queried (Table S2). Gene prediction of *S.* '*fitti*' assembly revealed 37,621 gene models, but many were incomplete (i.e., missing start or stop codon; Tables S2 and S3). In the gene family analysis, 807 orthogroups were found to be shared by the Symbiodiniaceae assemblies excluding *S.* '*fitti*', whereas 2,225 orthogroups were found to be shared by all Symbiodiniaceae assemblies (Figure S1; including *S.* '*fitti*'). Additionally, 202 orthogroups were uniquely shared by *S.* '*fitti*' and its closest relative *S. tridacnidorum* whereas 209 orthogroups were shared by the seven assemblies from the genus *Symbiodinium* (Figure S1).

3.2 | Genomic variation of *Symbiodinium* '*fitti*'

Based on the analysis of *S.* '*fitti*'-specific microsatellite loci, the majority of shallow-sequenced samples harboured one strain of *S.* '*fitti* (*n* = 47, 75.9% of *A. palmata*–*S.* '*fitti*' and 82.6% of *A. cervicornis*– and hybrid–*S.* '*fitti*'; Table S1) and were used for further analysis. A total of 2,505,230 SNPs and 569,337 indels were identified between all samples. Of these, 58,538 SNPs and 1874 indels were considered "high-quality" (Figure 1). The 58,538 "high-quality" SNPs represent a

range of per-SNP fixation levels from 0 to 1 (Figure S2). The average transition/transversion ratio of the "high-quality" SNPs was 1.79 and did not vary by host species (Table S1). In total, 16,536 of the "high-quality" SNPs (28.2%) occurred in coding regions (Table S4). BLAST reports indicated that the majority of the SNPs (87.4%) in coding regions matched other Symbiodiniaceae genomic resources (primarily the closely related species, *S. microadriaticum*). Multilocus genotype (MLG) filtering of the 58,538 "high-quality" SNPs indicated each sample represented a unique MLG (strain), consistent with the microsatellite analysis, and was retained for downstream analyses (Table S1). Additional filtering to remove variants with missing data resulted in 6,813 high-quality "conservative" SNPs. After this procedure of quality filtering SNPs and setting a stringent missing data threshold, the average read coverage increased from 1.53 to 11.3 per SNP (Table S1; average 655.3% increase).

3.3 | Patterns of host-specificity and biogeography within *S. 'fitti'*

The phylogeny of the *psbA* minicircle noncoding region revealed little differentiation between symbiont strains with respect to their host taxa, confirming that *S. 'fitti'* is one species (Figure S3). The AMOVA corroborated that most of the variation among *S. 'fitti'* is at the within-species level (86.1+%; Table S5). Variation attributed to host taxa explained 11.6% of the components of covariance (sigma =87.0) and then variation among the geographical location of each host explained 2.3% of the components of covariance (sigma =17.1; Table S5). Variation among geographical locations did not explain the components of covariance (−5.4%, sigma = −39.3) whereas variation among the host taxa at the various geographical locations explained 16.3% of the components of covariance (sigma =117.6; Table S5).

Consistent with the AMOVA results, samples clustered loosely by host taxon rather than geographical origins in a principal component analysis (PCA) with the "high-quality" SNPs and in the maximum likelihood tree with the conservative SNPs (Figures 2 and 3). In the PCA, 16% of the variance was explained by PC1 whereas 13.6% was explained by PC2 (Figure 2). Partitioning of *S. 'fitti'* to host taxon was recapitulated in a PCA with 5,067 selection outlier SNPs but not in a PCA of 16,536 gene region SNPs (Figures S4 and S5). No partitioning of *S. 'fitti'* by depth was observed for the subset of samples with depth data available (Figure S6; Table S1). Within each host taxon, there was some indication of biogeographical partitioning in the phylogeny but not in the PCA (Figures 2 and 3; Table S6). In the maximum likelihood tree, the *S. 'fitti'* associated with *A. cervicornis* formed a monophyletic clade (bootstrap support 78) separate from the *S. 'fitti'* associated with *A. palmata* and the hybrid that are polyphyletic (Figure 2). Analysis of STRUCTURE output using the delta *K* method (Evanno et al., 2005) identified three clusters as the most likely *K* (Table S6). The three clusters largely corresponded to host taxa (Figure 4). The differences between *A. cervicornis*–*S. 'fitti'* and *A. palmata*–*S. 'fitti'* SNPs corroborate the genomic variation between the two host species reported by Kitchen et al. (2019).

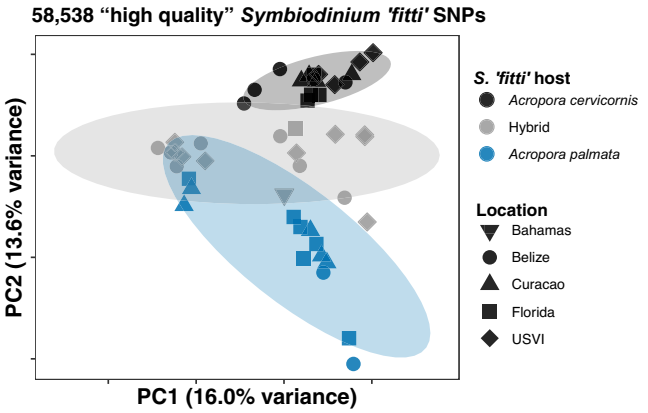

FIGURE 2 Principal component analysis (PCA) of 58,583 "high-quality" *Symbiodinium* '*fitti*' SNPs illustrates genomic differentiation by host taxon. Ellipses represent 95% confidence intervals for the multivariate distribution of each host taxon. Samples from different host taxa are denoted by point colour and point shape reflects geographical location [Colour figure can be viewed at wileyonlinelibrary.com]

3.4 | SNPs under selection

Of the "high-quality" SNPs, 4,987 (8.5%) were determined as selection outliers by PCADAPT (Figure 5). When BAYESCAN accounted for host identity, location of host, and host by location interaction, 217, five and 197 selection outliers were identified, respectively (n = 370 SNPs; Figure 5; Table S7). Additionally, 339 selection outlier SNPs were shared between the two programs (Figure 5; Table S7). In total, 103 outlier loci identified by BAYESCAN had a Bayes probability of 1 and q-value of 0 which becomes infinite following logarithmic transformation and were therefore removed from the Manhattan plot (Figure 5) but are reported in Table S7. For each set of SNPs under selection, a subset was found in coding regions (1453 from PCADAPT, 74 from BAYESCAN, 65 from both callers; Table S7).

3.5 | Predicted functional implications of SNPs within *S. 'fitti'*

Of the "high-quality" SNPs, SNPEFF identified 61,259 modifier/noncoding variants (82.9%), 4,670 moderate/mostly harmless variants (6.3%), 7825 low-impact variants that might change protein efficiency/effectiveness (10.6%), and 128 highly disruptive SNPs (0.17%). Of the predicted mutations, SNPEFF identified 4,692 nonsynonymous (missense) mutations (37.6%), 68 nonsense mutations (0.54%) and 7,718 silent mutations (61.9%; Table S8). The aforementioned mutations are predicted to cause 12,472 codon changes and 4,760 amino acid changes (Tables S8 and S9). In the 65 selection outlier SNPs in gene regions (shared by both callers), SNPEFF predicted 4053 downstream effects (Table S10). The majority (3,813/4,053) of these predicted effects occurred in an RNA-directed DNA polymerase from mobile element *jockey* (Table S10). SNPEFF also predicted other modifier (nonsynonymous) and low-impact mutations

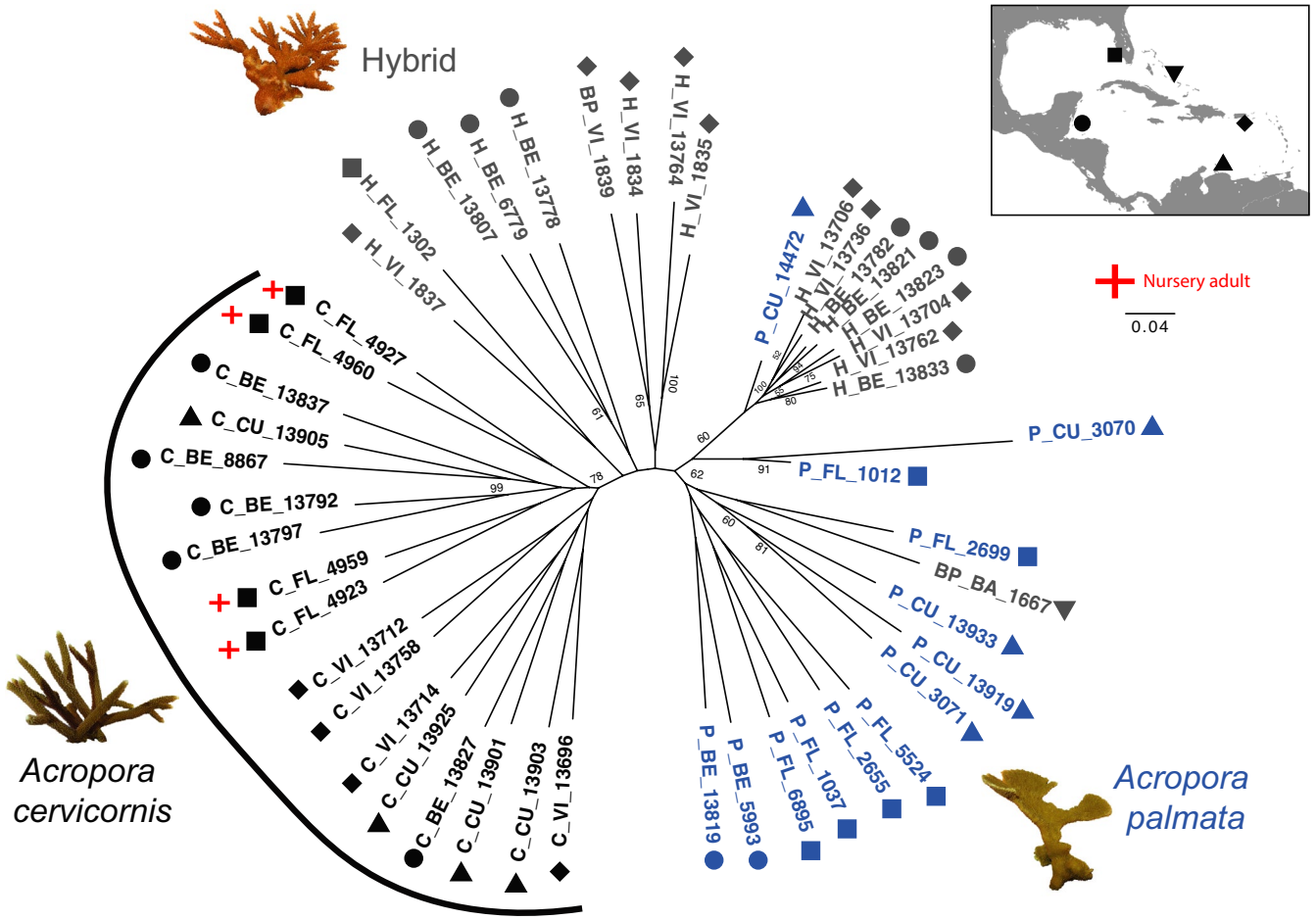

FIGURE 3 *Symbiodinium* '*fitti*' genomic variation is at the subspecies level. RAXML (maximum likelihood) phylogeny of 6,813 "conservative" genotyping *S.* '*fitti*' SNPs without missing data and 100 bootstrap replicates illustrate *S.* '*fitti*' genomic differentiation by host taxon. Samples from different host taxa are denoted by text and colour; point shape reflects geographical location. Four samples are adult acroporids from a coral nursery and the rest are reef-grown adults. Values denote bootstrap support (values <50 not shown). Coral images from N. Fogarty and I. Baums. Scale bar represents maximum likelihood estimation of substitutions per nucleotide site [Colour figure can be viewed at wileyonlinelibrary.com]

in peroxisomal adenine nucleotide carriers, ATPase RavA, nicotinamide phosphoribosyltransferase, phospholipid:diacylglycerol acyltransferase, uncharacterized proteins and their introns (Table S10).

4 | DISCUSSION

The population dynamics and evolutionary history of reef-building corals are relatively well studied compared to their endosymbiotic dinoflagellates. However, selection acts on both partners, and differences in life-history characteristics between dinoflagellates and corals suggest that the spatial and temporal scales of adaptation may differ. This study analysed SNPs of *Symbiodinium* '*fitti*' from the two Caribbean acroporid species and their F_1 hybrid. We show that the genomic variation of sympatric *S.* '*fitti*' populations is partitioned by host taxon (Figures 2–4) and describe two potential scenarios that would lead to this result. Differentiation of *S* '*fitti*' by host taxon supports coral–algal co-evolution, which is likely re-enforced by partner selectivity and recognition (Scenario 1). Alternatively, co-evolution and partner selectivity *per se* (i.e., via specific recognition of genetic variants among partners) may not explain the patterns of genomic variation. Differences in the environment (light, depth, nutrient availability) associated with the habitat preferences of the host taxa may contribute to the observed patterns of symbiont differentiation (Scenario 2). However, it is probably a combination of these mechanisms that drives the co-evolution of *S.* '*fitti*' strains within Caribbean acroporids. In either case, *S.* '*fitti*' genetic diversity is tied to that of its endangered hosts.

4.1 | Coevolution of *S.* '*fitti*' and Caribbean acroporids

Co-evolution is the process by which two interacting species reciprocally adapt to each other (*sensu* Janzen, 1980). The ~160 million years following the widespread adaptive radiation of stony corals and Symbiodiniaceae has allowed sufficient time for co-evolutionary processes to be detected in the fossil record, which

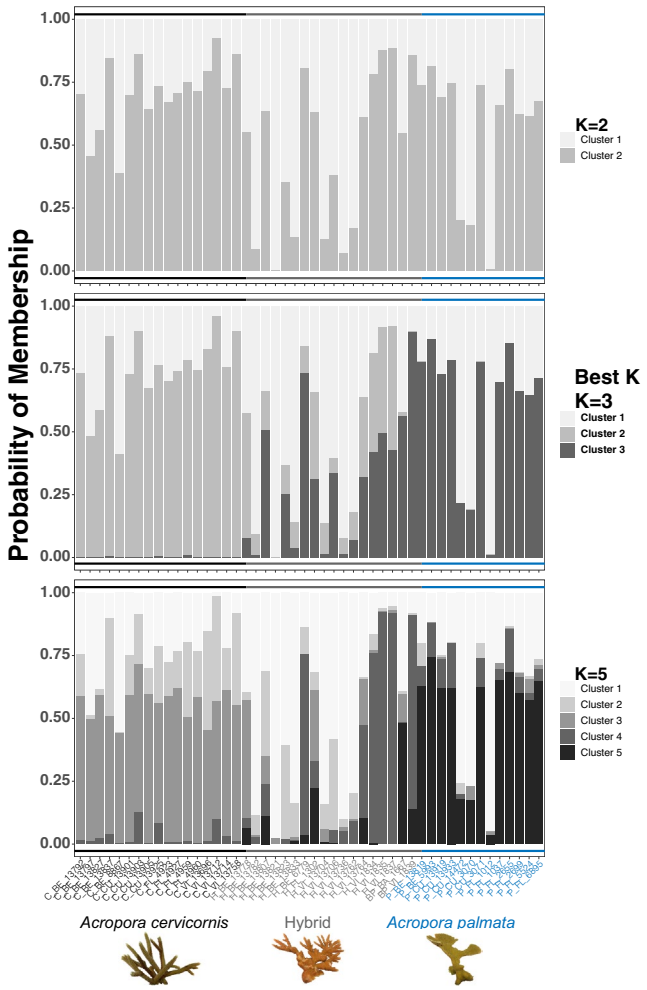

FIGURE 4 *Symbiodinium* '*fitti*' strain population assignment aligns with host taxon. Probability of membership predicted by STRUCTURE for 58,583 "high-quality" *S.* '*fitti*' SNPs. *K* = 3 was determined as the best *K*. *K* = 2 and *K* = 5 are also presented for comparison. These results illustrate that *S.* '*fitti*' membership clusters largely correspond to their host acroporid taxon. Samples from different host taxa are denoted by *x*-axis text colour. Coral images from N. Fogarty and I. Baums [Colour figure can be viewed at wileyonlinelibrary.com]

may explain partitioning of *S.* '*fitti*' by acroporid host species (Figures 2–4; LaJeunesse et al., 2018; Muscatine et al., 2005; Stanley, 2006). Cnidarian–dinoflagellate co-evolution has been observed in scleractinian corals, soft corals and anemones (Bellis et al., 2018; Forsman et al., 2020; Prada et al., 2014) and is corroborated by the long-term fidelity between partners across many other coral taxa (Goulet, 2006; Thornhill et al., 2006, 2014). Recent advances in phylogenomics found extensive differentiation of genomic features and gene family enrichment between symbiotic and free-living *Symbiodinium* spp., which also support co-evolution between Symbiodiniaceae and their hosts (González-Pech et al., 2017, 2019, 2021). Therefore, the adaptation of *S.* '*fitti*' to the intracellular environments associated with each acroporid host probably facilitated their co-evolution and the partitioning of genomic variation observed here (Figures 2–4; Figure S4).

Acropora rose to dominance in the fossil record relatively recently (~6–7 million years ago) and thus co-evolution between Caribbean acroporids and *S.* '*fitti*' has occurred on a short timescale (Mao et al., 2018; Renema et al., 2016; Wallace & Rosen, 2006). Therefore, the subtle clustering of genomic variation of *S.* '*fitti*' in relation to acroporid taxa may reflect recent co-evolution and, perhaps, the onset of speciation (Figures 2–4). In other words, signals of population genomic structure driven by coral host taxon are relatively weak in *S.* '*fitti*' compared to Symbiodiniaceae lineages that have already undergone speciation (Figures 2–4; Davies et al., 2020; Lewis et al., 2019; Wham et al., 2017). Interestingly, partitioning of *S.* '*fitti*' seems to be driven by selection outlier SNPs rather than gene region SNPs, but it is possible other genomic features contribute to the nonrandom sorting to host acroporids (Figures S4 and S5). In the remainder of the Discussion, we consider how the processes of partner selectivity and environmental differentiation may re-enforce *Acropora*–*S.* '*fitti*' co-evolution.

4.2 | Interpartner specificity and selectivity in a horizontally transmitting symbiotic mutualism

Specificity between Symbiodiniaceae and adult corals that acquire symbionts via horizontal transmission is commonly observed at the species level with multimarker and microsatellite approaches (Chan et al., 2019; LaJeunesse, 2001; Lewis et al., 2019). Despite the occasional presence of other genera of Symbiodiniaceae, the associations between *S.* '*fitti*' and Caribbean acroporids are stable through time (Baums et al., 2010; Thornhill et al., 2006). By showing the partitioning of *S.* '*fitti*' to its three Caribbean acroporid hosts, we reveal that partner specificity can be detected at an even finer (SNP) level which, to some extent, is driven by selection outliers (Figures 2–4; Figure S5).

The selection pressures that result in long-term fidelity between partners have yet to be pinpointed but probably involve cell-signalling and partner recognition mechanisms (LaJeunesse et al., 2018; Parkinson et al., 2018; Wood-Charlson et al., 2006). As a result, the symbiotic pairings have to meet the unique biochemical and metabolic demands of each host microenvironment in the long term (Barott et al., 2015; Sogin et al., 2014). Each acroporid taxon possesses unique skeletal morphologies and corallite structures, which results in variable light availability to the resident endosymbiont and thus may create a strong selection pressure for the associated *S.* '*fitti*' (Enríquez et al., 2017; Gladfelter, 1983, 2007). Ultimately, the extent to which selection pressures (cell signalling, biochemical, skeletal morphology) contribute to the specificity between *S.* '*fitti*' strains and their acroporid hosts will need to be quantified with future experimental work.

Akin to the role of host specificity, symbiont selectivity is also important for retaining partner fidelity in symbioses. Symbiodiniaceae that form mutualisms with reef-building corals only represent a portion of their total diversity and abundance in reef environments (Cunning et al., 2016; Decelle et al., 2018). As

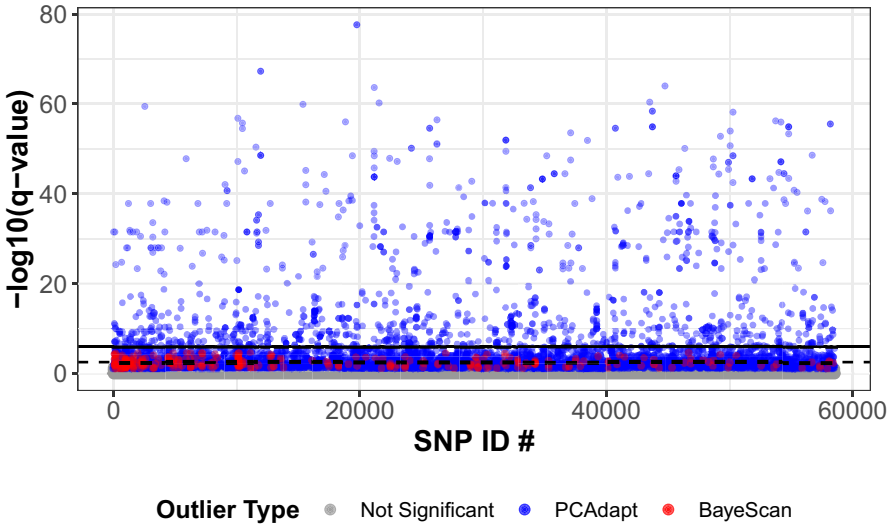

FIGURE 5 Genetic variants of *Symbiodinium* '*fitti*' showing signatures of selection. Manhattan plot of $-\log_{10}$=transformed *q*-values for 58,583 "high-quality" SNPs. SNPs are highlighted by outlier detection program (PCADAPT loci highlighted in blue; BAYESCAN loci highlighted in red). In total, 339 selection outlier SNPs were shared between the two programs. The 103 outlier loci identified by BAYESCAN with infinite $-\log_{10}$ q-values were removed from the plot. These loci also had high BAYESCAN fixation levels between host and location. The dashed line represents the 5% FDR adjustment threshold (2.54) whereas the solid line represents the 0.05 Bonferroni correction threshold (6.01) [Colour figure can be viewed at wileyonlinelibrary.com]

such, the diversity of *S.* '*fitti*' probably extends beyond strains that inhabit acroporids and may incorporate free-living conspecifics living in the water column, sediments, etc. Therefore, the slight differences in genomic variation of each *S.* '*fitti*' strain may be a result of host preference of the available symbiont strains (Figures 2–4). Conversely, symbiotic *S.* '*fitti*' strains might be attracted to the different microbial composition in the water column adjacent to a coral colony that constitute the "ecosphere" surrounding each acroporid (Weber et al., 2019). Putative intraspecific variation in the swimming availability and chemosensory responses of *S.* '*fitti*' may also, in part, dictate which Symbiodiniaceae persist in each ecosphere (Fitt, 1984, 1985; Fitt et al., 1981; Kamykowski et al., 1992). Future experimental validation of intraspecific variation in *S.* '*fitti*' swimming and chemosensory ability and how they pertain to selectivity of their acroporid hosts (and "ecospheres") will shed light on how interpartner specificity and coral–dinoflagellate co-evolution are established and maintained.

4.3 | The special case of the F_1 coral hybrid as habitat

The shared history (~2.6–3.6 million years of coexistence) between *A. palmata* and *A. cervicornis* with their symbiont *S.* '*fitti*' may have allowed sufficient time for co-evolution and the development of partner specificity (Figures 2–4; Budd & Johnson, 1999; McNeill et al., 1997). However, the situation differs for their first-generation hybrid which cannot directly respond to selection pressure from *S.* '*fitti*' via differential successful sexual reproduction of its colonies because an F2 is not formed (Vollmer & Palumbi, 2002). Thus, genomic variation of the hybrid is restricted to somatic mutations occurring within their lifetime which can be on the order of hundreds of years (Irwin et al., 2017). *S.* '*fitti*' may have encountered these colonies over many thousands of years as they are generated anew with each hybridization event. Immediately following the colonization of the hybrid, *S.* '*fitti*' are exposed to strong selection pressures driven by various aspects of the hybrid's biogeochemical and morphological properties (Enríquez et al., 2017; Sogin et al., 2014). Thus, while a host co-adaptive response is unlikely, *S.* '*fitti*' may have evolved strains that preferentially colonize the hybrid.

4.4 | Environmental differentiation as a driver of intraspecific genomic variation

S. '*fitti*' population dynamics are confounded with differences in host taxon habitat preferences including light, temperature, nutrient concentration and food availability in the water column (Crossland & Barnes, 1983; Miller, 1995; Terraneo et al., 2019; Williams et al., 2018). Light (and therefore depth) exerts strong selection pressure on microalgae and frequently leads to zonation of Symbiodiniaceae populations (Bongaerts et al., 2015; LaJeunesse, 2002; Serrano et al., 2014). Until recently, the three Caribbean acroporid taxa resided at different depths throughout their distribution (Adey et al. 1977; Cairns, 1982; Goreau, 1959; Rützler & Macintyre, 1982). Habitat differentiation among the three taxa is now less frequent and the hybrid generally grows near at least one parental species (Fogarty, 2012) and it is projected to become the dominant reef-building coral on some shallow Caribbean reefs (Nylander-Asplin et al., 2021). Though

it can be found alongside *A. cervicornis*, the hybrid often occupies a shallower depth range which tends to overlap with *A. palmata* (Fogarty, 2012). Adaptation to different light availabilities may contribute to genomic differentiation between the shallow paraphyletic "clade" of *A. palmata*–*S.* '*fitti*' and hybrid–*S.* '*fitti*' vs. the monophyletic "clade" of deep *A. cervicornis*–*S.* '*fitti*' (Figure 3; Figure S6; Table S1; Finney et al., 2010; Kirk et al., 2009). However, there was no subclustering of samples by depth (5–27 m) within the monophyletic "clade" of *A. cervicornis*–*S.* '*fitti*' so genomic differentiation is not solely due to depth (Figure S6; Table S1). The repeated observation of coral–dinoflagellate specificity spanning environmental gradients reported here and throughout the literature suggests that the genomic differentiation of Symbiodiniaceae is not entirely driven by environmental differences (Goulet, 2006; Thornhill et al., 2014). Future quantification and comparison of how Symbiodiniaceae respond to selection pressures created by external environmental differences (i.e., outside the host habitat) vs. those created by internal microenvironmental differences (i.e., inside the host) will improve our understanding of the ecology and evolution of symbiosis.

4.5 | Potential for gene region selection outliers to facilitate the adaptation of *S. 'fitti'* to the lifestyles associated with the microenvironments of host acroporids

Selection outliers, including those within gene regions, may underlie the partitioning of *S.* '*fitti*' genomic variation by host taxa (Figure S4, Tables S7–S10). Changes in amino acid sequences and protein efficiency resulting from these mutations may facilitate the adaptation of *S.* '*fitti*' to the microenvironments associated with each host (Tables S7–S10; Parkinson et al., 2015; Parkinson & Baums, 2014). The majority of shared selection outliers in gene regions were predicted to cause downstream effects in a transposable element (RNA-directed DNA polymerase from mobile element *jockey*; Table S10). It is possible that following activation, this transposable element catalyses further genomic variation, which has been observed in other microalgae (Chen et al., 2017; Maumus et al., 2009). Other shared outlier mutations occurred in genes related to ATP metabolism (Arai et al., 2008; Cross et al., 2000; Wong et al., 2017), NAD biosynthesis (Hashida et al., 2009) and phospholipid metabolism (Table S10; Ståhl et al., 2004). The predicted downstream effects of these mutations are likely to alter *S.* '*fitti*' metabolism and physiology (Tables S7–S10; Kmiec et al., 2016; Zhang et al., 2018). Subsequently, these changes may enable *S.* '*fitti*' to adapt to the unique metabolic and nutritional demands imposed by each host's microenvironment (Figures 2–5; Tables S7–S10; Muscatine et al., 1989; Reich et al., 2020; Sogin et al., 2014).

5 | CONCLUSION

We show here that the population genetic structure of *Symbiodinium* '*fitti*' is, in part, explained by its host taxon association. The large number of nonsynonymous SNPs differentiating the *S.* '*fitti*' strains among their host taxa may demarcate the onset of eventual speciation. That said, since the host taxa frequently occupy different habitats, we cannot fully disentangle the role of host vs. environment as a potential driver of population genetic structure. The genomic resources for the *S.* '*fitti*'–acroporid system described here can be used in future studies to determine whether, and to what degree, the observed genomic variation is a result of co-evolution, host selectivity, symbiont selectivity, environmental differentiation or a combination of these mechanisms. Thorough evaluation of population genetic structure and evolutionary dynamics of both coral holobiont partners will improve understanding of the genomic underpinnings of a coral's physiological capacity and ability to withstand climate change stressors.

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AUTHOR CONTRIBUTIONS

Conceived the project: S.A.K., I.B.B., H.G.R. Obtained funding: I.B.B., N.D.F. Mentorship: I.B.B., S.A.K. Field collections of corals: S.A.K., I.B.B., N.D.F. Molecular work: S.A.K., M.D.D. Bioinformatic analyses: H.G.R., S.A.K., K.H.S. Wrote the paper: H.G.R., S.A.K., I.B.B., N.D.F., K.H.S.

DATA AVAILABILITY STATEMENT

Sequences are available under NCBI SRA PRJNA473816. Code for data analysis and figure generation is available on github (https://github.com/hgreich/Sfitti). The draft genome assembly for the reference *A. cervicornis*–*S.* '*fitti*' is available on dryad https://doi.org/10.5061/dryad.xgxd254g8

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SUPPORTING INFORMATION

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

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