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Contrasting effects of *Symbiodinium* identity on coral host transcriptional profiles across latitudes

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Abstract

Reef-building corals can increase their resistance to heat-induced bleaching through adaptation and acclimatization and/or by associating with a more thermo-tolerant strain of algal symbiont (*Symbiodinium* sp.). Here, we show that these two adaptive pathways interact. We collected *Acropora millepora* corals from two contrasting thermal environments on the Great Barrier Reef: cooler, mid-latitude Orpheus Island, where all corals hosted a heat-sensitive clade C *Symbiodinium*, and warmer, low-latitude Wilkie Island, where corals hosted either a clade C or a more thermo-tolerant clade D. Corals were kept in a benign common garden to reveal differences in baseline gene expression, reflecting prior adaptation/long-term acclimatization. Model-based analysis identified gene expression differences between Wilkie and Orpheus corals that were negatively correlated with previously described transcriptome-wide signatures of heat stress, signifying generally elevated thermotolerance of Wilkie corals. Yet, model-free analyses of gene expression revealed that Wilkie corals hosting clade C were distinct from Wilkie corals hosting clade D, whereas Orpheus corals were more variable. Wilkie corals hosting clade C symbionts exhibited unique functional signatures, including downregulation of histone proteins and ion channels and upregulation of chaperones and RNA processing genes, putatively representing constitutive "frontloading" of stress response genes. Furthermore, clade C *Symbiodinium* exhibited constitutive expression differences between Wilkie and Orpheus, indicative of contrasting life history strategies. Our results demonstrate that hosting alternative *Symbiodinium* types is associated with different pathways of local adaptation for the coral host. These interactions could play a significant role in setting the direction of genetic adaptation to global warming in the two symbiotic partners.

1 | INTRODUCTION

Climate change is threatening the persistence of diverse ecosystems around the world; however, the effects of rising temperatures are particularly acute for coral reefs, which have experienced drastic declines in coral cover over the past several decades. When water temperatures exceed the thermal tolerance threshold of reef-building corals for a prolonged period of time (Brown, 1997), the endosymbiotic algae and/or their pigments are lost from the corals' tissue in a process known as bleaching. Sustained periods of high ocean temperatures can lead to extensive coral bleaching and subsequent mortality (Ainsworth et al., 2016; Great Barrier Reef Marine Park Authority, 2016). Natural variation in thermal regimes across reef habitats, however, can provide the framework for evolution of elevated coral thermal tolerance, based on local thermal adaptation and migrant exchange among populations (Dixon et al., 2015). Local genetic adaptation and/or long-term physiological acclimatization (henceforth jointly referred to as "adaptation" for short) have been shown to contribute to increased thermotolerance of a variety reef-building coral species in American Samoa, the Great Barrier Reef and

the Florida Keys (Bay & Palumbi, 2014; Dixon et al., 2015; Kenkel et al., 2013).

Reef-building corals can also form associations with more heat-tolerant strains of *Symbiodinium*, which thereby impart greater thermotolerance on the resulting coral-*Symbiodinium* association (Baker, Starger, McClanahan, & Glynn, 2004; Berkelmans & van Oppen, 2006; Rowan, 2004) termed the "holobiont". In particular, *Symbiodinium* from genotypic clade D typically increase the bleaching thresholds of the coral host relative to clade C on the GBR, are preferentially retained after bleaching events and are often found in high temperature and turbid reef environments (Ladner, Barshis, & Palumbi, 2012; Oliver & Palumbi, 2009, 2011; Stat & Gates, 2011). In addition, *Symbiodinium* within the same clade exhibit local adaptation to different environments (Baums, Devlin-Durante, & LaJeunesse, 2014; Levin et al., 2016; Oliver & Palumbi, 2011). Thus, multiple factors can operate together (host adaptation, symbiont adaptation and various combinations of host and *Symbiodinium*) to bring about elevated thermal tolerance of the holobiont. Here, we address whether these potential adaptive pathways interact. In a specific way, we test whether long-lasting molecular responses of the coral host to local conditions depend upon the clade, and potentially thermotolerance, of its symbiont. Answering this question is important for evaluating the potential for coevolution between coral and *Symbiodinium*, which is, in turn, essential for modelling the rates and spatial patterns of coral adaptation to global warming.

Thermal variation along the latitudinal extent of the GBR provides a natural setting in which to test for the effects of adaptation in corals populations and their photo-symbionts. Low-latitude regions in the northern GBR generally experience warmer summer temperatures than middle and high latitude regions, and also differ in their abundance and distribution of clade D *Symbiodinium*, which in part depends on temperature extremes and recent bleaching history (Cooper et al., 2011). To address the potential significance of variation in both thermal history and symbiont type (clade C vs. D) on adaptive physiological signatures, we collected corals (*Acropora millepora*) from two locations separated by 5 degrees of latitude along the GBR (low-latitude, warmer temperature Wilkie Reef and mid-latitude, cooler Orpheus Island) and maintained their replicate fragments in a common garden for 3 weeks under ambient Orpheus Island conditions (Figure 1). We then profiled genomewide gene expression in both the coral animal and *Symbiodinium* to identify origin-dependent signatures that have persisted despite 3 weeks in a common garden, which are putatively associated with local adaptation or long-term acclimatization to the corals' native habitats. We show that association with the same *Symbiodinium* clade leads to different physiological outcomes for the coral host depending on location, while genetically similar symbionts associated with the same host species but originating from different thermal environments exhibit differences in gene expression potentially indicative of differences in life history strategy (growth vs. sexual reproduction).

2 | METHODS

2.1 | Coral collection and TagSeq library preparation

In November 2014, nine colonies of *Acropora millepora* were collected from Wilkie Reef (13′78S; 143′64E) and eleven were collected from Orpheus Island (18′61S:146′48E) and brought to the Orpheus Island Research Station (Figure 1a–c). Samples collected from the same location were sampled haphazardly at approximately the same depth (3–5 m) on the reef flat facing inshore. These

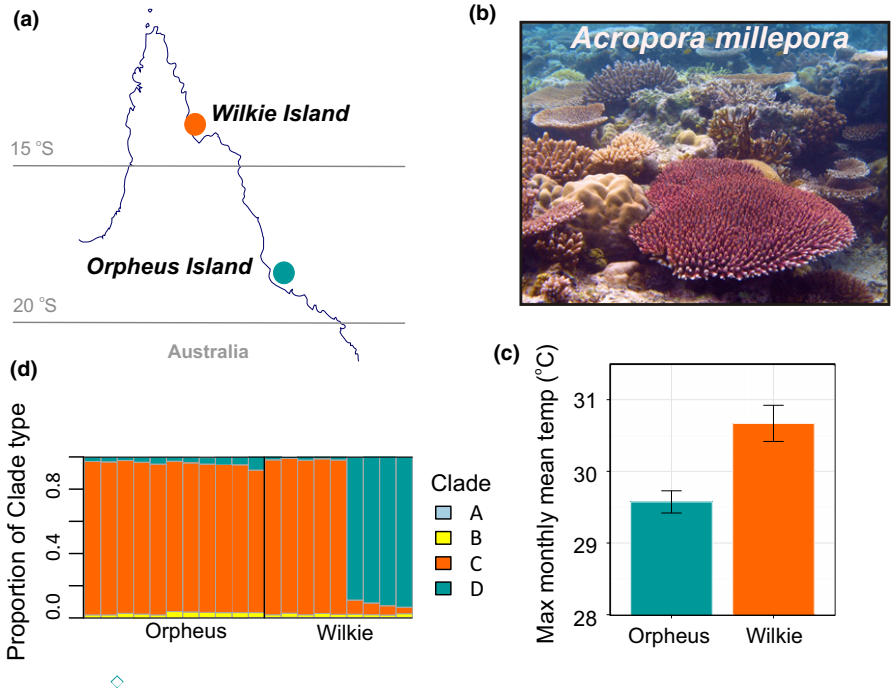

FIGURE 1 Study location and system. (a) Location of Orpheus and Wilkie Islands along the Great Barrier Reef, Australia. (b) Photograph of our focus species, *Acropora millepora*. (c) Maximum monthly mean temperature at Orpheus and Wilkie Islands. (d) Proportion of symbiont clade type for each genotype in this study. Bars represent different genotypes and colours are indicative of the *Symbiodinium* clade type

locations differ by about 1.5°C in the maximum monthly mean temperature throughout the year (Dixon et al., 2015), the main parameter determining the local thermal bleaching threshold (Donner, Stone, Allen, Liu, & Arzayus, 2009). Three fragments comprising 3–4 branch tips were split off each colony, yielding a total of 60 fragments. The fragments were assigned random positions in an outdoor circular raceway, such that no two replicate fragments were placed next to each other, and supplied with a steady flow of natural sand-filtered seawater at the ambient temperature (26.5–27°C midnight, 29–29.5°C midday) at the Orpheus Island Research Station. The fragments were kept in the raceway for 21 days under natural lighting reduced by 75% shade cloth. In a subsequent manner, a single branch tip from each fragment was preserved in 100% ethanol and kept at −20°C. RNA was isolated using RNAqueous kit (Ambion) and TagSeq (Meyer, Aglyamova, & Matz, 2011) libraries were prepared as described in the protocol available at https://github.com/z0on/tag-based_RNAseq. All libraries were sequenced on the Illumina HiSeq 2,500 platform. Sequences were adaptor-trimmed and de-duplicated based on the identity of 64-fold degenerate 5′-leader, length of oligo-G linker and identity of the first 30 bases of the read sequence. At last, the reads were quality-filtered to retain only reads containing more than 80% of bases with PHRED quality exceeding 20 using FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/).

2.2 | *Symbiodinium* clade type determination

Acroporid corals are able to associate with a large diversity of *Symbiodinium* types that mostly group into four major clades: A, B, C and D (Quigley et al., 2014; Rowan, 2004). To determine the predominant clade type in each coral colony, the TagSeq reads were mapped with BOWTIE v2.1.0 simultaneously to the coral transcriptome (Moya et al., 2012) and transcriptomes of *Symbiodinium* clades A–D. Transcriptomes for Symbiodinium clades A and B were from Bayer et al. (2012), and transcriptomes for clades C and D were from Ladner et al. (2012). We then counted the relative proportions of reads producing highly unique matches (mapping quality 40 or higher) to each *Symbiodinium* transcriptome, using a custom perl script zooxType.pl (Supporting information File S1).

2.3 | Differential gene expression analysis: host and symbiont

The method used for RNA extraction does not separate host and symbiont RNA prior to TagSeq library preparation. Thus, to obtain counts for each expressed gene, sequences were mapped simultaneously to the *A. millepora* transcriptome from Moya et al. (2012) and the *Symbiodinium* clades C and D transcriptomes generated from *Acropora hyacinthus* in Ladner et al. (2012) with BOWTIE v2.1.0. The per gene counts were derived by summing up the numbers of reads matching to all sequences representing the same gene, while reads mapping to multiple genes were disregarded. The resulting counts per sample ranged from 352,210 to 1,402,424, with a median of 570,211 reads mapping to the host transcriptome. The read counts that mapped to the symbiont transcriptome ranged from 103,529 to 175,450, with a median of 153,064. The R package DESEQ2 was used for subsequent differential expression analyses for both host and symbiont mapped reads. Three samples had low read counts for the host mapped data (O8a, W22a and W25c) and were excluded from the analyses, resulting in a total of 57 samples (includes replicates) distributed among 20 unique genotypes. For host gene expression, we fit a model with coral individual as a factor first and then performed contrasts based on the reef of origin and the dominant *Symbiodinium* clade hosted. As only Wilkie corals showed variation in symbiont clade type, three groups were contrasted: Orpheus corals (all clade C *Symbiodinium*), Wilkie corals with clade C *Symbiodinium* and Wilkie corals with clade D *Symbiodinium*. Hereafter, these sample groups are referred to as Orpheus C (O_C, 11 colonies, 31 fragments), Wilkie C (W_C, 5 colonies, 13 fragments) or Wilkie D (W_D, 4 colonies, 12 fragments). For clade C *Symbiondinium*, reads from replicate fragments of the same coral colony were combined, as fewer *Symbiodinium* reads were obtained in comparison to the host, and three corals (O8, O4 and OM1) were excluded because too few reads were recovered. This resulted in thirteen samples for the symbiont gene expression analysis. For both host and symbiont, a mean of two reads per gene was used as the threshold for retaining the gene for analysis, yielding a total of 19,547 genes for the hosts and 11,411 genes for the symbionts. The number of significantly differentially expressed genes (DEGs) at the FDR cut-off of 10% was determined with the R package empiricalFDR.DESeq2 (Wright, Aglyamova, Meyer, & Matz, 2015). Principal coordinate analysis of variance-stabilized gene expression was performed with the "VEGAN" package in R using Manhattan distances between samples, corresponding to the sum of log-fold differences across all genes (Dixon, 2009). The function adonis (R package VEGAN) was used for a permutational multivariate analysis (999 permutations) with Manhattan distances to determine if our predefined gene expression groups (O_C, W_C, W_D for host and Wilkie, Orpheus for symbiont) were significantly different from each other.

2.4 | Weighted gene coexpression network analysis

As an additional analysis to identify groups of genes that differentiate host location and symbiont type, we performed a weighted gene coexpression network analysis or weighted gene coexpression network analysis (WGCNA; Langerfeld & Horvath, 2008), which identifies groups of genes showing correlated expression across samples ("modules") in an unbiased fashion, without considering experimental conditions. Only genes with an adjusted *p*-value <0.1 were retained for the analysis, resulting in a total of 6,138 genes. A mean dissimilarity threshold of 0.5 was used to merge similar modules. We examined each module's eigengene expression (first principal component of all genes in the module) in individual coral colonies, for which we formed 20 pseudo-quantitative traits—vectors of numbers, one per sample, that were either "1" or "0" depending on whether or not the same came from a specific coral colony. The sign and strength of the correlation between the module's eigengene and this vector

(Figure 2b) indicate to what extent the module is up- or downregulated in the specific coral. Coexpression modules were then tested for functional enrichment with a twofold test implemented within the GO_MWU package (flag "isModule=TRUE"): transcriptome-wide Fisher's exact test for GO term enrichment among in-module genes, followed by within-module Mann–Whitney *U* test for GO term enrichment with genes highly correlated with the module's eigengene (false discovery rate is determined by permutations).

2.5 | Functional enrichment tests: GO Mann–Whitney *U* Test and KOG meta-analysis

To identify significant functional differences among the genes up and downregulated among the three host group comparisons (W_C vs. O_C, W_C vs. W_D and W_D vs. O_C) and between *Symbiodinium* populations, we used a Gene Ontology (GO) enrichment analysis that utilizes the Mann–Whitney *U* (MWU) test, as described in (Wright et al., 2017). The main advantage of this methodology is that it includes the entire ranked list of genes, instead of those chosen by an arbitrary significance cut-off, to determine whether certain GO categories contain significantly more up- or downregulated genes than expected by chance. *p*-values were multiplicity adjusted using the Benjamini–Hochberg method. Only adjusted *p*-values are reported. In addition, we employed a Eukaryotic Orthologous Group (KOG) MWU test (R package KOGMWU) to determine if there are significant differences in gene expression regulation among a set of 23 nonoverlapping KOG categories (Dixon et al., 2015). For the KOG analysis, additional gene expression data sets from adult and larvae of *A. millepora* that were subjected to heat stress were included as a comparison to our data (Meyer et al., 2011).

Due to the large number of genes in many KOG categories, those groups that were significantly enriched with up/downregulated genes were further broken down into subcategories based on individual gene annotations. This allows us to determine whether a small

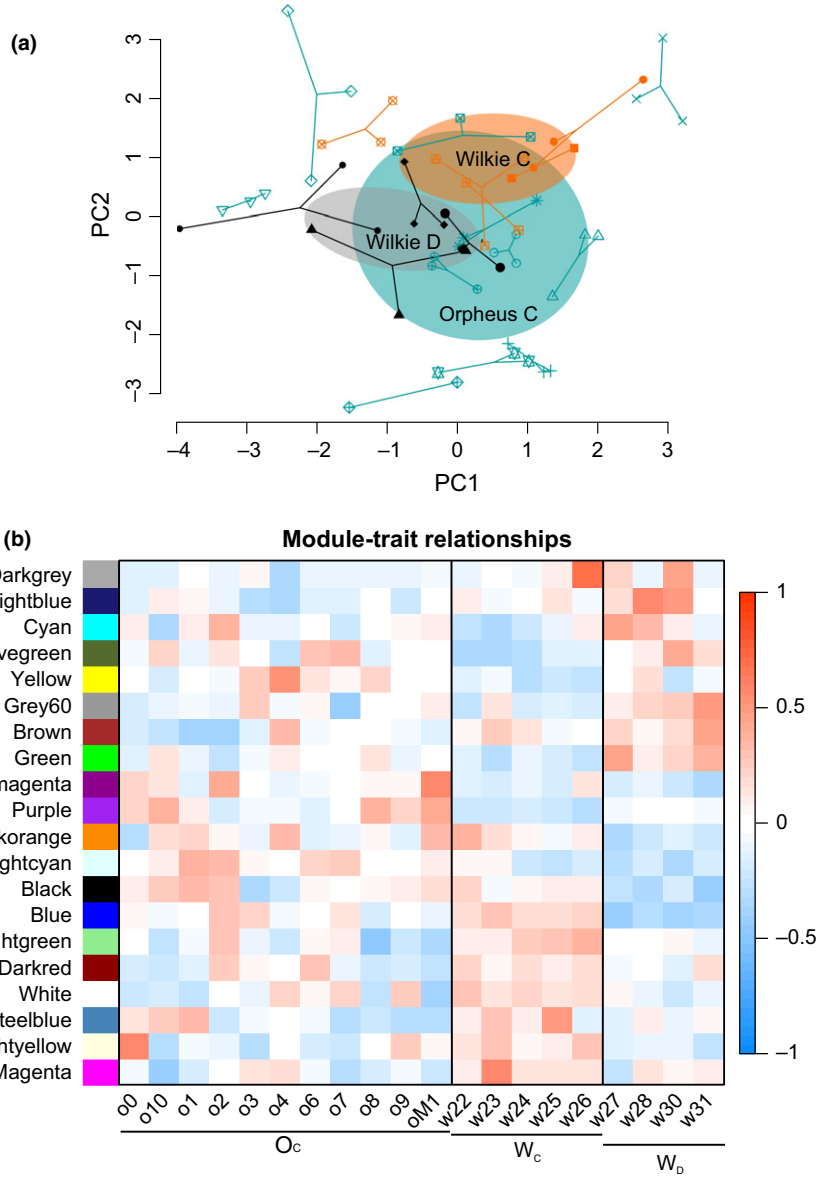

FIGURE 2 Analysis of similarity among coral host gene expression profiles for Orpheus C, Wilkie C, and Wilkie D corals. (a) Projection of principal coordinates 1 and 2 for host gene expression. (b) Weighted gene coexpression network analysis (WGCNA): correlations between traits (genotypes or sample groups) and modules (groups of genes with common expression patterns). Modules are indicated by colours to the left of the heatmap. Within the heatmap, red indicates positive correlation between a module's expression and gene expression in a trait of interest, blue indicates a negative correlation. Columns represent separate coral individuals or groups (W_C, W_D, O_C) [Colour figure can be viewed at wileyonlinelibrary.com]

number of subfunctions are driving expression patterns among certain KOG categories or whether it is an overall broad modulation of expression among many different gene functions.

2.6 | Genotyping of coral host and *Symbiondinium* clade C

Gene expression differences between populations could be driven by long-term acclimatization or genetic differences. To assess the degree of genetic divergence among either coral host or *Symbiodinium* clade C samples, TagSeq reads (pooled by colony) were mapped to the corresponding reference transcriptome (Ladner et al., 2012; Moya et al., 2012) using BOWTIE v2.1.0. Identity-by-state (IBS) distance matrices were generated using ANGSD with filters -minMapQ 20 -minQ 20 -snp_pval 1e-2 -minMaf 0.1. SNPs were required to be genotyped in at least 15 host samples and in at least 12 symbiont clade C samples, which retained 11,378 variants for the host and 877 variants for the symbiont (Korneliussen, Albrechtsen, & Nielsen, 2014). The IBS matrices were analysed using function *adonis* (package VEGAN) to determine statistical significance or separation of samples in multivariate space according to specified group structure (O_C, W_C and W_D). IBS-based ordination plots were generated using function capscale (package VEGAN) without specifying any constraints, which is equivalent to principal coordinate analysis. F_{ST} between Wilke and Orpheus *Symbiodinium* clade C was computed in ANGSD using the empirical site frequency spectrum as a prior, following the tutorial at https://github.com/mfumagalli/ngsTools/blob/master/TUTORIAL.md. In addition, analysis with ADMIXTURE was performed to identify any potential *Symbiodinium* clade C divergence within sites (Alexander, Shringarpure, Novembre & Lange, 2015).

3 | RESULTS

3.1 | Genetic divergence

The coral hosts did not show significant genetic divergence either between Orpheus and Wilkie or between O_C, W_C and W_D (Supporting information Figure S1A). While *Symbiodinium* clade C demonstrated significant (permutation test $p = 0.003$) divergence between Wilkie and Orpheus, (Supporting information Figure S1B), F_{ST} value associated with this divergence was only 0.014, indicating that *Symbiodinium* C from the two islands represent very closely related populations. No further groups or subgroups were identified beyond the distinction between Wilkie and Orpheus. ADMIXTURE corroborated this finding, as the recommended K was 1 and at $K = 2$, separation was between Orpheus and Wilkie. Thus, there are no cryptic *Symbiodinium* species at these sites.

3.2 | Symbiont type and Reef of Origin effects on host gene expression

Within Wilkie Reef, four individuals (W26, W27, W30 and W31) hosted primarily a clade D *Symbiodinium* whereas the other five Wilkie individuals and all Orpheus individuals hosted primarily a clade C *Symbiodinium* (Figure 1d). For all subsequent gene expression analyses of hosts, we used a generalized linear model of contrasts between the three groups: W_C, W_D and O_C. Principal coordinate analysis of the variance-stabilized gene expression data show that expression profiles of W_C and W_D genotypes form distinct clusters, separated on both principal coordinates one and two, which explain 8.2% and 6.4% of the variation, respectively (Figure 2a, b). Based on a permutational multivariate analysis, each of the three host groups can be considered statistically significantly distinct ($p < 0.001$).

With a Benjaimini–Hochberg FDR cut-off of 10%, the number of differentially expressed genes (DEGs) in the W_C vs. O_C, W_D vs. O_C and W_C vs. W_D comparisons was 3,094, 3,120 and 3,217 genes, respectively (Figure 3, Supporting information File S2). The W_C vs. W_D comparison contained the greatest number of uniquely differentially expressed genes (1,372) that were not shared with any other comparison, whereas the W_C vs. O_C and W_D vs. O_C comparisons only contained 779 and 945 unique DEGs, respectively.

3.3 | Weighted gene coexpression network analysis

To explore whether W_C, W_D and O_C exhibit group-level expression patterns that are not driven solely by a few individuals or genotypes within the group, we employed a weighted gene coexpression network analysis or WGCNA. In other words, how cohesive are the expression patterns among these three groups? Based on the correlations between the modules (groups of coregulated genes) identified by WGCNA and each coral, it is apparent that both W_C and W_D exhibit expression patterns that are similar across all genotypes in the group (Figure 2b). For example, the blue module (containing 406 genes) is consistently downregulated across all W_D genotypes and upregulated across all W_C genotypes. In contrast, there are no modules that are correlated in a consistent fashion across all O_C genotypes.

We also performed a GO enrichment analysis to determine if there were any gene functional categories associated with the

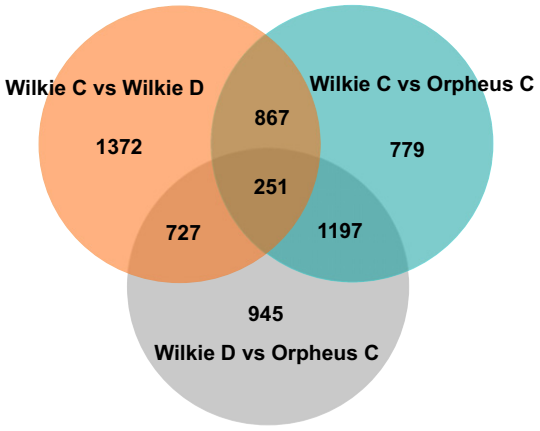

FIGURE 3 Venn diagram of the number of differentially expressed genes passing a 10% FDR cut-off for the three comparisons: Wilkie C vs. Wilkie D, Wilkie C vs. Orpheus C, and Wilkie D vs. Orpheus C [Colour figure can be viewed at wileyonlinelibrary.com]

modules identified by WGCNA. Of all 19 modules that were tested, only four were enriched with a GO term within either Molecular Function (MF), Biological Processes (BP), Cellular Components (CC) or a combination of these. Several of these GO terms were associated with ribosomal proteins and the ribonucleoprotein complex. The purple module, however, was enriched for extracellular matrix genes ($p_{adj} < 0.001$), and the magenta module was enriched for "cellular response to stress" ($p_{adj} = 0.08$) (Figure 2b, Table S1).

3.4 | Functional differences among host gene expression patterns

3.4.1 | Gene ontology analysis

To further explore functional differences between each of the three coral groups, we used GO enrichment analysis with the Mann–Whitney *U* test. Among the biological processes and cellular components, ribosome biogenesis was significantly ($p < 0.01$) enriched with upregulated genes in Wilkie corals relative to Orpheus corals (Figure 4), while extracellular matrix proteins and noncanonical Wnt signalling were downregulated in Wilkie corals ($p < 0.05$).

Biological processes and molecular functions that are enriched with upregulated genes exclusively in the W_C vs. O_C comparison include RNA binding ($p < 0.1$) and RNA and ncRNA metabolic processing ($p < 0.05$, $p < 0.1$), whereas developmental process ($p < 0.05$) is downregulated in W_C relative to O_C. Several GO terms were exclusively significant in the W_C vs. W_D comparison, including ion transmembrane transporters ($p < 0.1$), fucosidase ($p < 0.1$), S-methyltransferase ($p < 0.1$), nucleosome assembly ($p < 0.01$) and protein-DNA complex ($p < 0.01$), which are all downregulated in W_C (Figure 4).

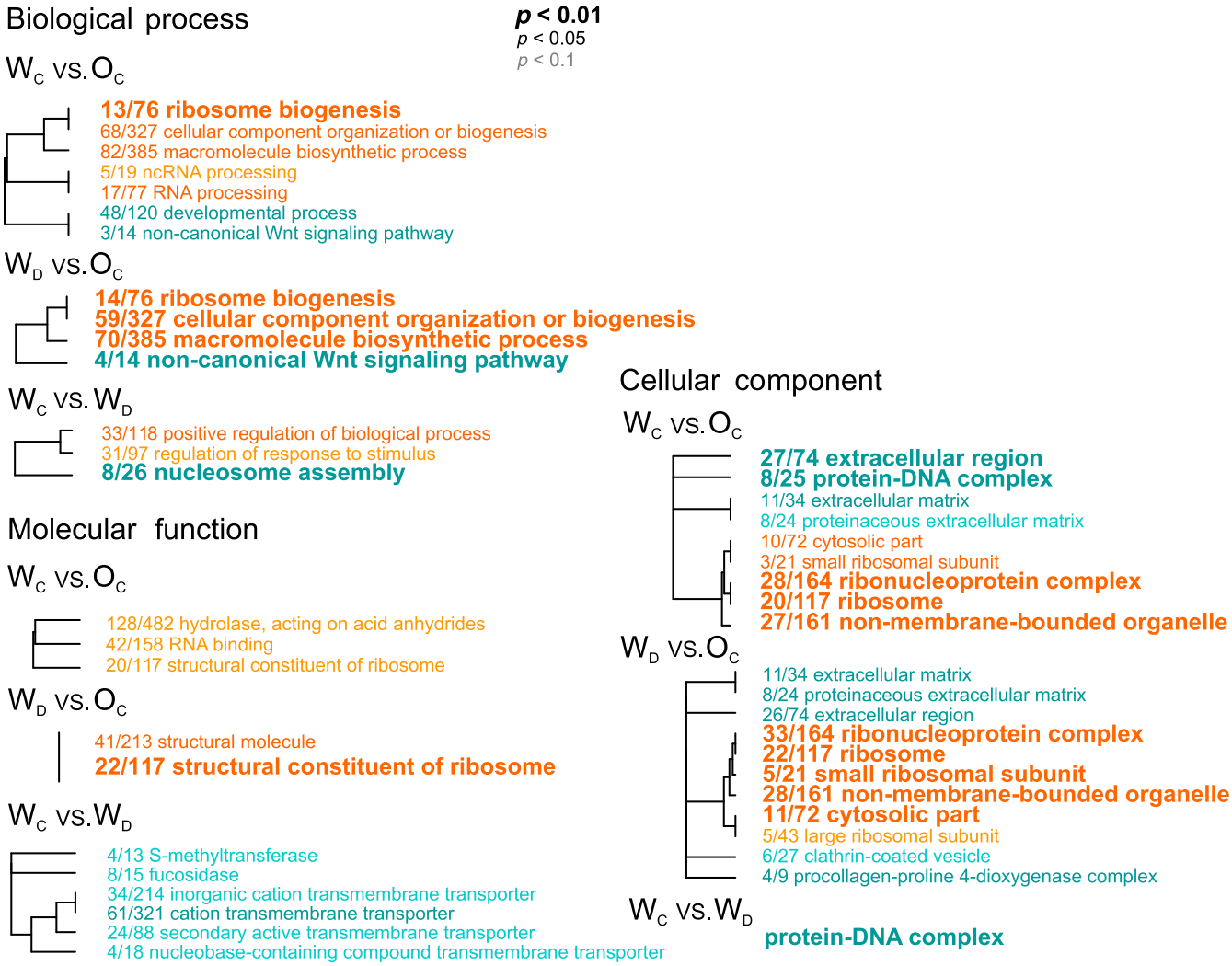

FIGURE 4 GO categories significantly enriched with up/downregulated genes based on Mann–Whitney *U* test. Orange indicates greater upregulation in a particular category, whereas blue indicates greater downregulation. Smallest font is equivalent to a *p*-value <0.1, medium font is a *p*-value <0.05, and large font is a *p*-value <0.01. Dendrograms represent hierarchical clustering of GO categories based on shared genes in the analysed data set [Colour figure can be viewed at wileyonlinelibrary.com]

3.4.2 | Eukaryotic orthologous group (KOG) meta-analysis

A Eukaryotic orthologous groups (KOG) analysis was used to identify broad categories of biological processes being modulated in each host group and to compare these data to published adult and larval *A. millepora* heat stress data sets (Meyer et al., 2011). The gene expression data sets used in the KOG meta-analysis were prepared in the same manner as ours, using tagSeq. In the experimental set-up of Meyer et al., *A. millepora* larvae from a single full-sibling family were subjected to either 4 hr or 5 days of heat stress at 31.4°C. Similar to that, adult *A. millepora* were subjected to 3 days of heat stress at 31.5°C and were then sampled for gene expression (Dixon et al., 2015).

Both W_C and W_D significantly upregulated genes related to translation, ribosomal structure and biogenesis and downregulated extracellular structure genes relative to O_C (Figure 5a). W_C corals were upregulating RNA processing and modification genes (p_{adj} = 0.002) and downregulating inorganic ion transport and metabolism genes (p_{adj} = 0.004) relative to O_C, whereas W_D corals were downregulating carbohydrate transport and metabolism (p_{adj} = 0.1) and upregulating replication, recombination and repair (p_{adj} = 0.1) relative to O_C. Several patterns seen in Wilkie relative to Orpheus are opposite to the patterns seen in the heat stress data set, most notably the categories translation/ribosomal structure and biogenesis, inorganic ion transport and metabolism, energy production and conversion, cell wall/membrane/envelope biogenesis and nuclear structure. Although the KOG delta ranks for both the W_C vs. O_C and W_D vs. O_C comparisons were not significantly correlated with the long-term heat stress (5 days) in larvae, they were significantly negatively correlated with the short-term larval heat stress data set (p = 0.00073 and p = 4.9e−5, respectively); and additionally, the W_C vs. O_C comparison (but not W_D vs. O_C) was significantly negatively correlated with adult heat stress data set (p = 0.022) (Figure 5b–g). However, the W_C vs. W_D comparison was not significantly correlated with any of the heat stress data sets (Figure 5h–j).

To further explore expression differences among the KOG functional categories, we subcategorized genes by their functional annotation within significant KOG categories, including ion transport and metabolism, extracellular structures, and RNA processing and modification (Supporting information Figures S2–S5). Of 23 subcategories for ion transport and metabolism (Supporting information Figure S2), only one category (sulphate/bicarbonate/oxalate exchanger (Tukey HSD, p = 0.026)) was significantly downregulated in W_C relative to O_C compared to W_D relative to O_C, and one additional category was nearly significant (inward rectifier K^+ channel, p = 0.097). However, several subcategories are downregulated in both W_C and W_D compared to Orpheus, including Ca^{2+} channels, Ca^{2+}/Na^+ exchangers, Na^+ symporters, Ca^{2+}/Mg^{2+} cation channels, copper and Zn^{2+} transporters and sulphur-transferases.

Within RNA processing and modification, several subcategories of 44 showed greater upregulation in W_C relative to O_C than in W_D

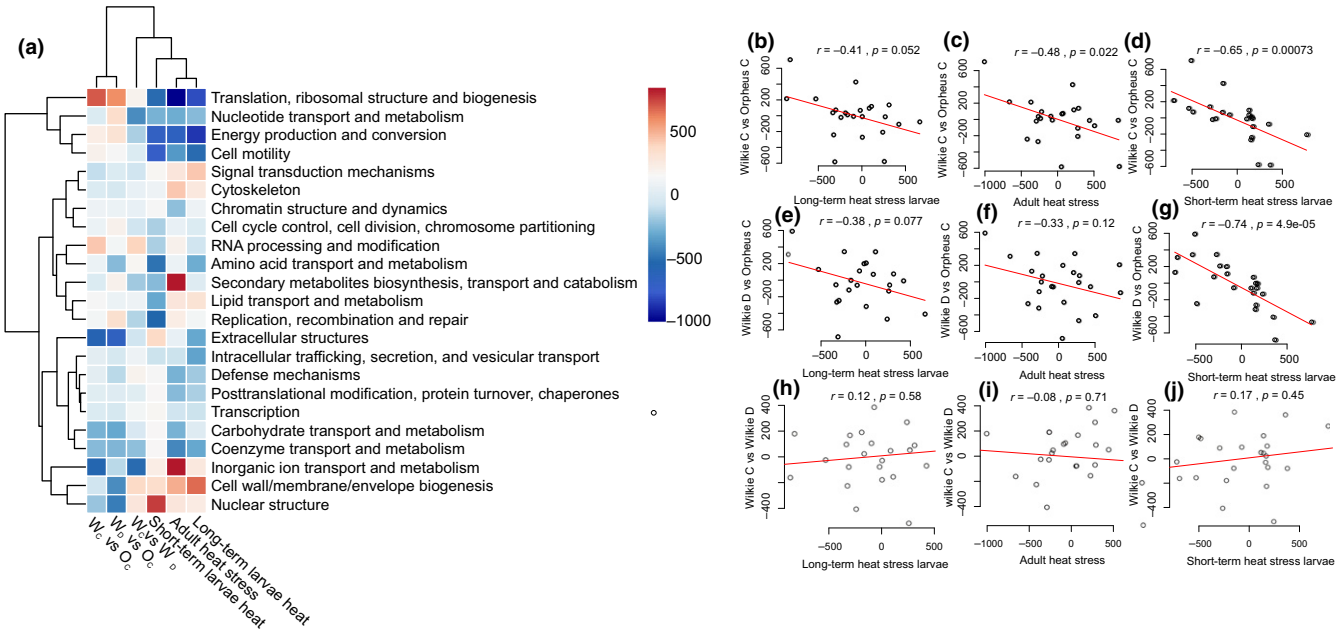

FIGURE 5 Eukaryotic orthologous group (KOG) meta-analysis. (a) Heat map showing KOG categories up- (red) and downregulated (blue) among the three host group comparisons (W_C vs. W_D, W_C vs. O_C, and W_D vs. O_C), as well as short and long-term larval heat stress and adult heat stress in *A. millepora*. (b) Correlation between KOG delta ranks for W_C vs. O_C and long-term larval heat stress, (c) W_C vs. O_C and adult heat stress, (d) W_C vs. O_C and short-term larval heat stress, (e) W_D vs. O_C and long-term larval heat stress, (f) W_D vs. O_C and adult heat stress, (g) W_D vs. O_C and short-term larval heat stress, (h) W_C vs. W_D long-term larval heat stress, (i) W_C vs. W_D and adult heat stress, and (j) W_C vs. W_D and short-term larval heat stress. The heatmap scale and *X*-axis are the delta rank values, which is a measure of how strongly a category is enriched by either up or downregulated genes in a particular data set [Colour figure can be viewed at wileyonlinelibrary.com]

relative to O_C, including many processes related to splicing (Supporting information Figures S3 and S4). Extracellular structures showed broad and general downregulation in both W_C and W_D compared to O_C (Supporting information Figure S5).

3.4.3 | Expression of chaperones and heat shock proteins

As chaperones and heat shock proteins are well known to contribute to increased heat tolerance in a variety of organisms, we decided to examine evidence for differential expression of these proteins in the coral hosts. Of the four Hsp90 genes that were significantly differentially expressed ($p_{adj} < 0.1$), two genes show a pattern whereby W_C has the highest expression level relative to both W_D and O_C and the other two are highest in both W_C and W_D compared to O_C (Supporting information Figure S6). The most significantly differentially expressed gene among the four ($p_{adj} = 9.1 \times 10^{-6}$) has the highest relative expression in W_C. Similar to that, 4/5 Hsp70 genes have the highest expression in W_C (Supporting information Figure S6). Among DnaJ chaperones, 9/16 have the highest expression in W_C compared to both W_D and O_C; 3/16 have the highest expression in O_C; 2/16 are highest in W_D, and 2/16 are highest in both W_D and O_C (Supporting information Figure S7). Furthermore, the DnaJ gene with the highest absolute expression level is the most strongly upregulated in W_C.

3.5 | Clade C *Symbiodinium* expression patterns

To better understand adaptation in the coral holobiont, we additionally undertook a *Symbiodinium* centric analysis of our data. Clade D *Symbiodinium* were excluded from these analyses, as this group is too genetically divergent from clade C to confidently establish sequence homology at all but the most conserved genes. Principal coordinate analysis of variance-stabilized gene expression data from Wilkie and Orpheus symbionts shows a clear separation between the populations (Figure 6a). A permutational multivariate analysis of variance using distance matrices to determine whether Wilkie and Orpheus samples were significantly distinct yielded a *p*-value of 0.013. Between Wilkie and Orpheus symbionts, 52 DEGs were differentially expressed at a 10% FDR. Among these DEGs are genes involved in translation, chloroplast genes and meiosis-related genes (Figure 6b). In an interesting manner, 60S ribosomal protein is downregulated in the Wilkie symbiont samples, whereas they were upregulated in Wilkie C host samples (Figure 4). Neither GO analysis with

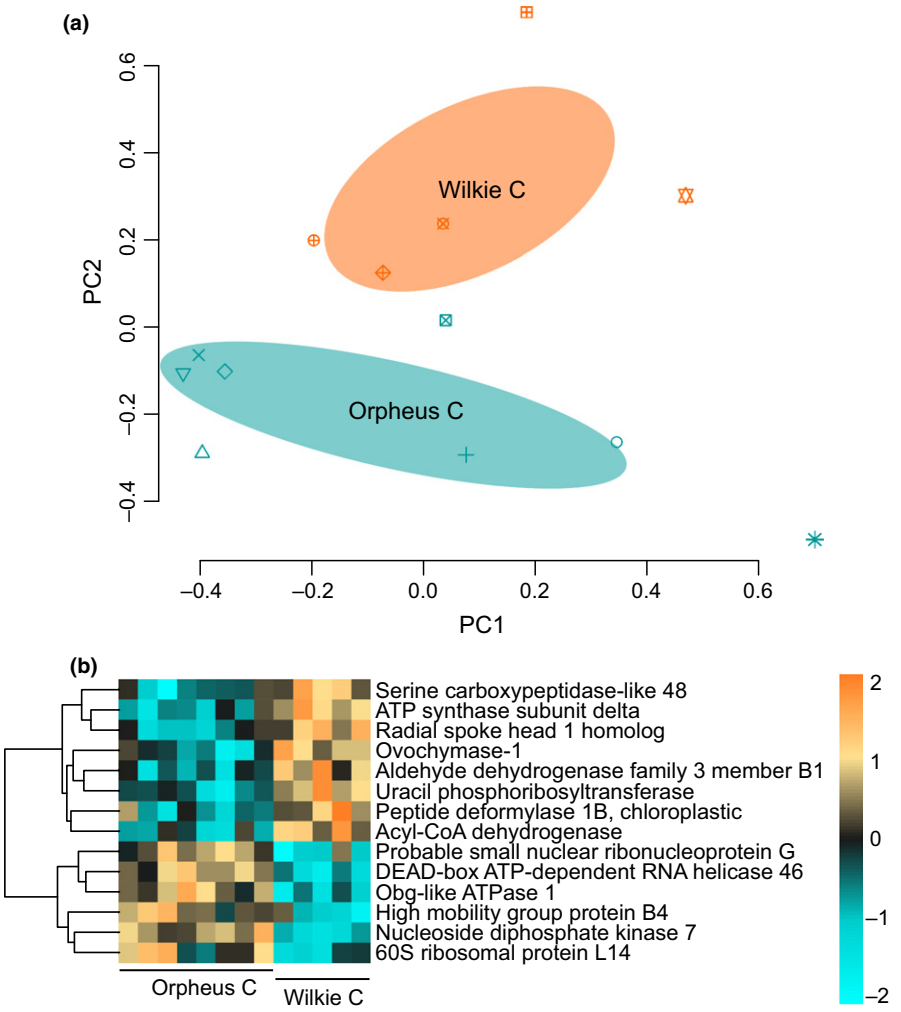

FIGURE 6 *Symbiodinium* clade C gene expression. (a) Principal coordinate analysis of symbiont gene expression from Wilkie C and Orpheus C origin corals. (b) Differentially expressed genes between Orpheus C and Wilkie C origin symbionts passing a 10% FDR cut-off [Colour figure can be viewed at wileyonlinelibrary.com]

MWU nor KOG analysis yielded significant results, likely owing to the small sample size.

4 | DISCUSSION

4.1 | Population level gene expression differences between Wilkie and Orpheus coral hosts

Significant differences in the transcriptomic profiles of W_C, W_D and O_C corals indicate that both native reef environment and symbiont clade modulate host physiology, as evidenced by stable shifts in baseline gene expression that persisted over 3 weeks in a common garden (Figure 2). Gene expression signatures shared among the Wilkie corals in comparison with Orpheus suggest that a host's local environment can affect certain aspects of physiology independent of symbiont clade type (Figures 4 and 5a). Most notably, Wilkie corals are significantly upregulating ribosomal proteins implying elevated ribosome production, a classic indicator of higher growth rate (Elser et al., 2003; Vrede, Dobberfuhl, Kooijman, & Elser, 2004). In addition, our KOG meta-analysis showed significant negative correlations between gene expression among Wilkie samples and previously published data from heat-stressed *A. millepora* larvae under short-term heat stress (Figure 5b–g), although only W_C relative to O_C was significantly negatively correlated with the long-term larvae and adult heat stress data sets (Figure 5b, c, $p = 0.05$, $p = 0.02$). We also observed no significant correlation between the any of the KOG values from the heat stress data sets and the KOG values from the W_C vs. W_D comparison, demonstrating that W_C and W_D exhibited equally low levels of stress (Figure 5h–j). In a wide fashion, this could indicate that Wilkie corals overall were experiencing less stress than Orpheus corals in the common garden experiment, even though it was carried out at the Orpheus Island Research Station in a raceway with flow-through local water. A plausible explanation of this is that the temperature of the common garden (28°C) was closer to the summer maximum temperatures for Orpheus corals than for Wilkie corals.

In addition, KOG subcategories within Inorganic Ion Transport and Metabolism that included Ca^{2+} cation channels were downregulated in both W_C and W_D. In the past, downregulation of calcium binding domains and channels has been associated with adult response to heat stress in corals and may be indicative of changes in calcification (Meyer et al., 2011). Our results suggest that either downregulation of calcium channels is a persistent response to high temperatures that did not change in the common garden over a 3-week period; or this could be a more general response among corals to changes in their environment.

Within the KOG category extracellular structures, several subcategories, including collagens, matrix metalloproteases, syntrophins, teneurins, mucins and nidogen are downregulated in Wilkie corals, suggesting that these corals could be changing their mucus production as a result of the change in their environment (Supporting information Figure S5) (Brown & Bythell, 2005; Jatkar et al., 2010). Increased expression of these proteins has previously been associated with both pCO_2 stress and heat stress in corals (DeSalvo et al., 2010; Moya et al., 2012; Seneca & Palumbi, 2015), and thus, decreased expression in Wilkie relative to O_C could be a general indication of reduced background stress levels.

Based on the host genotyping results, there does not appear to be significant genetic differentiation between Wilkie and Orpheus corals, nor O_C, W_C or W_D (Supporting information Figure S1A). This suggests that acclimatization could contribute significantly to adaptive gene expression differences.

4.2 | Symbiont clade affects host strategies for living in a thermally stressful environment

As all Wilkie corals exhibited gene expression signatures indicative of reduced background stress levels compared to Orpheus corals (Figures 4 and 5a), it is possible that corals from this location (both W_C and W_D) are resilient to high temperatures, even if they host different symbiont clades with different predicted thermotolerances. If this is the case, W_C and W_D hosts may utilize different adaptive strategies to handle their native high temperature environment, depending on whether their *Symbiodinium* have high predicted heat tolerance (D) or low predicted heat tolerance (C). One way for W_C to compensate for lower thermotolerance in their symbionts is "frontloading" sensu Barshis et al. (2013), which is pre-emptive upregulation of stress response genes. In support of this hypothesis, Hsp70 is one of the most strongly upregulated genes in W_C compared to W_D and O_C (Supporting information Figure S6). Upregulation of this gene has been associated with increased thermotolerance in a broad array of taxa, including insects, birds, plants and crustaceans (Al-Zghoul et al., 2013; Bedulina et al., 2013; Dong, Ji, Meng, Dong, & Sun, 2010; Gehring & Wehnert, 1995; Guo et al., 2016; Krebs, 1999) and was also identified by Barshis et al. (2013) as a frontloaded gene that contributed to increased thermal resilience in *Acropora hyacinthus*. Of significantly differentially expressed genes involved in protein folding ($p_{adj} < 0.1$), Hsp90, DnaJ and Hsp70 chaperones on average all have higher median baseline expression in W_C compared to W_D and O_C (Supporting information Figures S6 and S7). We also found that the magenta WGCNA module (Figure 2b) is significantly enriched with genes annotated with the GO term "cellular response to stress" ($p_{adj} = 0.08$). This module tends to be upregulated in W_C corals compared to O_C and W_D, strengthening the evidence that W_C corals are compensating for decreased holobiont thermal resilience compared to W_D corals via "front-loading" thermal stress-responsive genes, in particular protein-folding chaperones.

In addition, the KOG profile of W_C corals is significantly negatively correlated with both the adult and long-term larval heat stress KOG profiles, whereas W_D corals do not have a significant correlation with either of those data sets, although there is a trend in the same direction (Figure 5b–g). Potentially, this suggests W_C hosts have shifted their baseline transcriptional state as a strategy for long-term survival in a warm environment, given the predicted lower thermal tolerance of their symbionts. In an alternative way, the

transition to the common garden could have resulted in a more pronounced transcriptional reaction to the cooler environment in W_D corals. This could explain why the W_D vs. O_C comparison was very significantly negatively correlated with the short-term heat stress larval data set ($p = 4.9 \times 10^{-5}$). However, the W_C vs. O_C comparison was nearly as significant ($p = 0.00073$), and, thus, this could just be a signature that occurs in Wilkie corals irrespective of their symbiont type when placed in a less thermally challenging environment. We also found that there was no significant correlation between the KOG profiles of W_C vs. W_D and the heat stress data sets, suggesting that neither W_C nor W_D is more stressed with respect to each other.

Another signature that differed between W_C and W_D was downregulation of inorganic ion transport and metabolism in W_C (Figure 5a). Heat stress in corals can lead to cellular ionic imbalances that disrupt osmolyte transfer between hosts and their photo-symbionts, potentially leading to photoinhibition, generation of reactive oxygen species, bleaching and even apoptosis (Mayfield & Gates, 2007). Seibt and Schlichter (2001) demonstrate that a compatible intracellular ion concentration of host cells is needed for efficient photosynthesis by symbionts. The "Inorganic ion transport and metabolism" KOG category is one of the most strongly upregulated under heat stress in adult *A. millepora* (Figure 5a) and strongly downregulated in heat-tolerant *A. millepora* larvae (Dixon et al., 2015), suggesting this could be a signature of diminished overall stress or enhanced stress tolerance in corals. Only a few ion transport subcategories, however, (Supporting information Figure S2) are downregulated to a greater extent in W_C compared to W_D and O_C, including inward rectifying K+ channels, concentrative Na+ nucleoside, sulphate/bicarbonate/oxalate exchanger, divalent cation tolerance proteins and tandem pore domain K+ channels. Thus, specific ion transport functions, rather than broad expression modulation of ion channels, may contribute to a diminished stress signature or potentially even enhanced stress resilience in W_C.

Studies by Kenkel and Matz (2016) and Parkinson, Banaszak, Altman, LaJeunesse, and Baums (2015) support the notion that hosts actively modify their physiology to minimize stress in the symbiont. The signatures we find in W_C corals suggest that they are up/down-regulating certain genes as a potential stress buffer, which in turn could result in a better functioning symbiont under adverse conditions. However, the signature does not seem to apply broadly to the transcriptome, as only certain gene functions are differentially regulated in W_C compared to either O_C or W_D. From Barshis et al, for example, their "front-loading" response is restricted to a small subset of the *A. hyacinthus* transcriptome, suggesting that it is indeed a limited transcriptional reaction to stress in the environment, albeit with potentially significant effects on fitness. Furthermore, the strong negative correlations between Wilkie corals' expression compared to Orpheus corals and gene expression under heat stress agree with previous work that corals adapted to live in high temperatures have lower baseline levels of physiological stress (Barshis et al., 2013; Dixon et al., 2015).

4.3 | Alternative symbiont types result in distinct transcriptional signatures in corals from Wilkie Reef

Although W_C, W_D and O_C are significantly distinct groups based on MANOVA of gene expression, our principal coordinate analysis demonstrates that O_C is a much less cohesive group compared to W_C and W_D. Although we considered that O_C could be less cohesive due to harbouring multiple symbiont species or populations, we could not identify any distinct genetic groups within Orpheus symbionts based on principal coordinate analysis with genotyped SNPs (Supporting information Figure S1B). Results from WGCNA agree with this conclusion, as W_C and W_D exhibit similar gene expression patterns across all genotypes in several modules (Figure 2b). In contrast, there are no modules that are correlated in same direction across all genotypes for O_C. In addition, among the significant differentially expressed genes in the three comparisons, W_C relative to W_D contained the greatest number of unique genes (i.e., not shared with any other comparison) differentially expressed (1,372 vs. 779 and 945) (Figure 3), which supports the notion that there are more genes involved in the interaction between symbiont types, specifically in a warm environment, than between corals from different thermal environments. This could also explain why there are more consistent correlations across genotypes in the WGCNA modules in W_C and W_D. Inhabiting an environment in the upper thermal limit for corals, Wilkie individuals need to be locally adapted and/or acclimatized to survive; and/or they can associate with a symbiont type offering enhanced thermal tolerance. In either case, there may be greater selection pressure to maintain adaptive gene expression responses to the environment than in a cooler location like Orpheus. Given the ample evidence to support higher heat tolerance of clade D symbionts relative to clade C in the GBR (Baums et al., 2014; Levin et al., 2016; Oliver & Palumbi, 2009, 2011), it seems plausible that environmental stressors, such as heat or light stress, could produce greatly different adaptive responses depending on host-symbiont type. Our gene expression data do indeed show that there are unique baseline expression signatures in the host depending on symbiont type; but specifically, this pattern is most apparent in corals originating from a warm population. Strong signatures of differential gene expression depending on symbiont type in corals, as we have shown, have also been demonstrated in DeSalvo et al. (2010) and suggest that symbiont type may exert a greater influence on the host physiological state than even thermal stress. Our analysis extends this idea by suggesting that symbiont type is a vital determinant of how the coral host acclimatizes to its environment.

Our analyses of baseline gene expression in W_C and W_D show key differences in the repertoire of genes being expressed, potentially indicating important adaptive or physiological signatures distinguishing the groups. Most notably, the KOG category "RNA processing and modification" is significantly upregulated in W_C compared to both W_D and O_C; and the GO terms RNA processing ($p < 0.05$) and ncRNA processing ($p < 0.1$) are significantly upregulated in W_C relative to O_C (Figure 5a). Within subcategories for the KOG term "RNA processing and modification," a diversity of gene

functions, such as splicing, RNA and polyadenylate binding, and ribonucleoproteins, show large, albeit nonsignificant, differences in expression between W_C and W_D (Supporting information Figures S3 and S4). Many of these gene functions are implicated in post-transcriptional modifications of RNA, which can affect splicing, localization and translation of mRNA (Mata, Marguerat, & Bahler, 2005). In a wide fashion, this suggests that the host response to alternative symbiont types may be mediated not only through expression changes, but also through post-transcriptional modifications. For example, Baumgarten et al. (2017) demonstrate that microRNAs, involved in post-transcriptional regulation, may be important for maintaining host–symbiont relationships in Cnidarians.

Other baseline gene expression differences include upregulation of histones in W_D and to a lesser extent in O_C in reference to W_C. These histone genes are classified in the GO terms nucleosome assembly ($p < 0.01$) and protein-DNA complex ($p < 0.01$) (Figure 4). Upregulation of histones is a well-established marker of an increased cell division rate (Gunesdogan, Jackle, & Herzig, 2014). W_D corals are also upregulating S-methyltransferases ($p < 0.1$), which are involved in histone modifications (Cedar & Bergman, 2009), and Alpha-L-fucosidases ($p < 0.1$) (Supporting information Figure S8), a gene function that has previously been identified as a candidate for adaptation to thermally extreme environments in corals (Bay & Palumbi, 2014).

In contrast to W_C and W_D, there are few expression signatures in O_C that indicate functional differences, besides downregulation of ribosomal proteins and higher expression of extracellular proteins (Figure 4). This result may reflect the fact that Orpheus colonies exhibit more variable gene expression patterns overall and overlap to some extent with both C and D dominated colonies from Wilkie based on the principal coordinate analysis of gene expression (Figure 2a). The source of this variation is currently unclear, but could be a potential result of the central location of Orpheus Island within the GBR (Figure 1a), where it can receive migrants from genetically distinct northern and southern populations (Ayre & Hughes, 2004). An alternative hypothesis is that the milder conditions at Orpheus have resulted in less constrained baseline expression patterns compared to individuals from Wilkie, whose expression may be more constrained by acclimatization to a thermally stressful environment.

4.4 | In hospite Clade C *Symbiodinium* exhibit transcriptomic and genetic differences between coral hosts from different reef environments

Significant differences in the transcriptomic profiles of Clade C symbionts from Wilkie and Orpheus reefs indicate that either genetic divergence (Figure 1b), putatively involving adaptation to local environment, or acclimatization *in hospite* can result in constitutive gene expression differences among symbiont populations (Figure 6a). Based on the symbiont genotyping results, clade C *Symbiodinium* from Wilkie and Orpheus show low but significant genetic divergence ($F_{ST} = 0.014$), and, thus, the gene expression differences between them could partly have a genetic basis. Contrary to our findings, previous gene expression studies in *Symbiodinium* that have examined the influence of variable environmental conditions have found little transcriptomic change, at least relative to the host (Barshis, Ladner, Oliver, & Palumbi, 2014; Palumbi, Barshis, & Bay, 2012). This could indicate overall less transcriptomic plasticity in *Symbiodinium* compared to coral hosts, or that gene expression changes are likely to occur in *Symbiodinium in hospite* only after longer acclimation periods. However, Levin et al report notable differences in gene expression responses at 32°C between two clade C populations that differ in their thermotolerance with some similarities to our results (Levin et al., 2016). We found an upregulation of meiosis-related genes in the Wilkie C symbionts (ovochymase-1 and radial spoke head homolog 1; Figure 6b), which was also reported in Levin et al. (2016) for the thermo-tolerant population after 9 days at 32°C. This result was interpreted in light of the adaptive benefits of engaging in sexual reproduction in stressful environments. Another response we observe in W_C symbionts is downregulation of ribosomal proteins and genes related to cell division and growth, which are upregulated in W_C hosts and vice versa for O_C symbionts and hosts. This may indicate potential symbiotic regulatory interactions among ribosomal and growth-related proteins that affect the growth of host and symbiont. These results could also indicate that Orpheus and Wilkie symbionts are employing alternative life history strategies, whereby Orpheus symbionts are investing more in vegetative growth and Wilkie symbionts are investing more in sexual reproduction. In addition, Wilkie symbionts are upregulating energy production and chloroplast genes, such as ATP synthase, which could indicate overall more robust photosynthetic function and reduced stress compared to Orpheus symbionts within our common garden conditions (Figure 6b).

5 | CONCLUSIONS

We highlight novel interactions between the coral host and its *Symbiodinium* clade that influence location-specific gene expression in both partners. Our results indicate that molecular mechanisms of local adaptation in both corals and their symbionts are highly interdependent. In the coral hosts, alternative symbiont types produce distinct transcriptional responses even in a common garden, highlighting the considerable influence that this partnership has on the overall physiological state of the host. This has important implications for evolution of the coral-*Symbiodinium* association under global warming: alternative partnerships open up additional ways for each partner to be thermo-tolerant, which amplifies the variation that natural selection can act upon to evolve a thermo-tolerant coral holobiont.

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AUTHOR CONTRIBUTIONS

S.J.B analysed the data and prepared the manuscript. M.V.M. and L.K.B. designed the study, conducted the fieldwork and provided feedback on the manuscript. G.V.A. prepared the tagSeq samples for sequencing.

DATA ACCESSIBILITY

All scripts and data for the study are available at https://github.com/sbarfield/tagSeq-commonGarden.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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