Coral Reefs
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REPORT

Multi-omic characterization of mechanisms contributing to rapid phenotypic plasticity in the coral *Acropora cervicornis* under divergent environments

Javier A. Rodriguez-Casariego[1,4] · Alex Mercado-Molina[2] · Leila Soledade Lemos[3] · Natalia Soares Quinete[3] · Anthony Bellantuono[5] · Mauricio Rodriguez-Lanetty[5] · Alberto Sabat[4] · Jose M. Eirin-Lopez[1]

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Abstract Phenotypic plasticity is defined as a property of individual genotypes to produce different phenotypes when exposed to different environmental conditions. This ability may be expressed at behavioral, biochemical, physiological, and/or developmental levels, exerting direct influence over species' demographic performance. In reef-building corals, a group critically threatened by global change in the Anthropocene, non-genetic mechanisms (i.e., epigenetic and microbiome variation) have been shown to participate in plastic physiological responses to environmental change. Yet, the precise way in which these mechanisms interact, contribute to such responses, and their adaptive potential is still obscure. The present work aims to fill this gap by using a multi-omics approach to elucidate the contribution and interconnection of the mechanisms modulating phenotypic plasticity in staghorn coral (*Acropora cervicornis*) clones subject to different depth conditions. Results show changes in lipidome, epigenome and transcriptome, but not in symbiotic and microbial communities. In addition, a potential shift toward a more heterotrophic feeding behavior was evidenced in corals at the deeper site. These observations are consistent with a multi-mechanism modulation of rapid acclimation in corals, underscoring the complexity of this process and the importance of a multifactorial approach to inform potential intervention to enhance coral adaptive capacity.

Keywords Staghorn coral · WGBS · Transcriptome · Lipidome · Epigenetics · Phenotypic plasticity · Microbiome · Symbiodiniaceae

Introduction

The persistence of coral reef ecosystems will depend on the capacity of stony corals to acclimatize and adapt to a rapidly changing environment (Hoegh-Guldberg et al. 2017). Sustained anthropogenic disturbances to the planet's climate (The Royal Society and National Academy of Sciences 2014), shifts in ocean chemistry (Feely et al. 2009; Regnier et al. 2013), and biological organization and ecosystem inherent processes (Doney et al. 2012; Poloczanska et al. 2013; Rosenzweig et al. 2008), have caused dramatic declines in coral cover and regime shifts in many reefs worldwide (Hoegh-Guldberg et al. 2017; Robinson et al. 2019; van Woesik et al. 2022). Such an alarming scenario has elicited the interest to study mechanisms with the potential to produce rapid acclimatization and adaptation responses in corals (Eirin-Lopez and Putnam 2019; Hackerott et al. 2021).

Phenotypic plasticity serves as a base for selection to act upon (Yanagida et al. 2015) enabling organisms to rapidly modulate different physiological, morphological

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✉ Jose M. Eirin-Lopez
jeirinlo@fiu.edu

1 Environmental Epigenetics Laboratory, Institute of Environment, Florida International University, Biscayne Bay Campus, 3000 NE 151 Street, Office MSB-360, North Miami, FL 33181, USA

2 Sociedad Ambiente Marino, San Juan, PR, USA

3 Department of Chemistry and Biochemistry, Institute of Environment, Florida International University, Miami, FL, USA

4 Department of Biology, University of Puerto Rico, Río Piedras, San Juan, PR, USA

5 Department of Biological Sciences, Florida International University, Miami, FL, USA

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and behavioral traits (phenotype) in response to environmental change (Bonamour et al. 2019). If adaptive, this plasticity facilitates population survival under rapid environmental change until genetic adaptation can occur, and even when non-adaptive, plasticity can result in rapid adaptive evolution (Ghalambor et al. 2015). Considering the life history traits of stony corals (i.e., sessile, long lifespan), mechanisms enhancing phenotypic plasticity may be critical for their long-term survival (Payne and Wagner 2018). Epigenetic modifications generating heritable alternative gene activity states from the same DNA sequence (Cavalli and Heard 2019) have been shown to participate in responses to environmental change in marine invertebrates, including corals (reviewed in Eirin-Lopez and Putnam 2019). In addition, substantial transcriptional plasticity has been observed in corals facing different types of environmental stress (Bellantuono et al. 2012; Bay and Palumbi 2015; Brener-Raffalli et al. 2022; Kenkel and Matz 2016; Rivera et al. 2021; Traylor-Knowles et al. 2017), including the accumulation of stress-related transcripts (e.g., heat shock proteins) in anticipation of a recurrent stress episode (also known as "frontloading"). Yet, while there is some evidence of epigenetic modulation of gene expression in corals (Baumgarten et al. 2018; Dixon et al. 2018; Li et al. 2018), detailed mechanistic knowledge is still lacking, notably as it refers to the direct causal correlation between DNA methylation and differential gene expression (Dixon and Matz 2022; Rodriguez-Casariego et al. 2022). Overall, the crosstalk between epigenetic mechanisms regulating gene expression (Li et al. 2018; Weizman and Levy 2019) and the transient and non-deterministic nature of epigenetic marks make such a mechanistic association difficult (Adrian-Kalchhauser et al. 2020; Rodriguez-Casariego et al. 2022).

As metaorganisms composed by a multitude of functionally connected members (Ainsworth et al. 2020), ecological, genetic, epigenetic and physiological changes occurring in any of the components of the coral holobiont (i.e., dinoflagellates, bacteria, viruses, fungi, archaea) or in their interaction with the host can encompass significant effects for the phenotype of the colony (Del Campo et al. 2017; van Oppen and Blackall 2019). The present work builds on this knowledge to further study the mechanisms involved in coral acclimatory responses using a multi-omics approach. The obtained results reveal significant changes in phenotype, gene expression, DNA methylation and lipidic composition across different environmental conditions, but not in the microbial community. These observations are consistent with a multi-mechanism modulation of rapid acclimation in corals.

Materials and methods

Study sites and experimental design

A detailed description of the study sites, experimental design, coral collection, and host genotyping can be found in (Rodríguez-Casariego et al. 2020). Briefly, a total of $n = 200$ naturally fragmented colonies of the staghorn coral (*Acropora cervicornis*) were collected in the aftermath of hurricanes Irma and Maria. Fragments were subdivided into two similarly sized ramets ($n = 400$) and directly outplanted into experimental plots at two depths (5 and 15 m) in neighboring reefs at Culebra Island, PR (Luis Peña reef—LP: 18°18′45.0"N, 65°20′08.4"W and Carlos Rosario reef—CR: 18°19′30.2"N, 65°19′52.7"W). Initial colonies ($n = 200$) were genotyped using 6 microsatellite loci (Baums et al. 2005), resulting in $n = 81$ genets (Rodríguez-Casariego et al. 2020).

Demographic performance of outplants

Survival of each individual fragment (alive, dead, or missing) across all reefs and depths was assessed monthly. Fragments were considered alive if they showed at least ~ 10% of total initial area with live tissue remaining, and a censoring strategy was used to construct life tables including missing fragments (mortality event was not observed) during the experiment.

Phenotypic and molecular datasets

For the physiological and molecular analyses in this study, a subset of the 200 initial colonies ($n = 13$) (Rodríguez-Casariego et al. 2020), with ramets surviving at both experimental depths for one year in Luis Peña reef, were monitored and sampled ($n = 26$ fragments). This reef was chosen given increased survival of fragments outplanted and the availability of detailed environmental datasets along the study period (Rodríguez-Casariego et al. 2020). Such a dataset includes depth-associated differences in temperature, dissolved oxygen, pH, and salinity (Table S1).

Sample collection, DNA and RNA extraction

Branches of ~ 5 cm in length were collected from each fragment ($n = 26$) one-year post-outplanting. Coral tissue was immediately flash frozen in liquid nitrogen and stored at −80℃. DNA and RNA were purified from flash-frozen tissue using the Quick DNA/RNA Mini-Prep kit (Zymo Research, Irvine, CA) as in (Rodríguez-Casariego et al. 2020). To produce DNA and RNA extracts appropriate for microbial community, and to limit contamination with symbiont nucleic acids on samples destined for host omics

analyses, "hard" and "soft" nucleic acid extraction strategies were applied to each coral sample. Briefly, ~ 100 mg of tissue, previously powdered in liquid nitrogen, was resuspended in 2 mL vials containing 500 mg of Zirconia/Silica beads (0.5 mm diameter) and 1 mL of DNA/RNA Shield (Zymo Research, Irvine, CA) in duplicate. For the "soft" extraction, coral host cells were gently lysed using two pulses of 30 s in a vortex, leaving most of the symbiont cells intact and thus enriching host DNA and RNA (Rodríguez-Casariego et al. 2020). For "hard" extraction, complete tissue homogenization was achieved with a Mini Bead Beater (BioSpec, Bartlesville, OK, USA), ensuring the representation of all components of the holobiont. After centrifugation (12,000×*g* for 5 min), continued following the kit manufacturer's instructions. RNA was extracted following the kit's protocol but only for the set of samples subject to "soft" homogenization, including in-column DNAse treatment. DNA and RNA quality was assessed via gel electrophoresis and spectrophotometric analysis (NanoVue GE Healthcare, Chicago, IL) as described elsewhere (Rivera-Casas et al. 2017). Concentrations were measured using a Qubit 2.0 Fluorometer (Thermo Fisher, Waltham, MA). RNA quality and purity were additionally assessed using a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) before library preparation.

Microbiome analyses

The Internal Transcribed Spacer 2 (ITS2) region of nuclear ribosomal DNA of the symbionts was used to identify changes in the algal symbiotic community using previously described primers (Pochon et al. 2001). Genomic DNA from "hard" extractions ($n = 26$) was shipped to the Genomic Sequencing and Analysis Facility at University of Texas at Austin for amplification, library preparation and sequencing on an Illumina® MiSeq (Illumina, California, USA), with a read length configuration of 2 × 250 bp. Symbiodiniaceae community composition was analyzed using the SymPortal Pipeline (Hume et al. 2019).

Bacterial composition was evaluated from amplicon libraries constructed for all samples ($n = 26$) by amplifying the V3-V4 16S region. Primers 341F and 805R with Nextera XT-compatible overhangs (TCGTCGGCAGCGTCA GATGTGTATAAGAGACAG**CCTACGGGNGGCWGC AG** and GTCTCGTGGGCTCGGAGATGTGTATAAGAG ACAG**GACTACHVGGGTATCTAATCC**, respectively, with the locus-specific portion of the oligonucleotide underlined) were used to amplify bacterial 16S through PCR. Following quality control using BioAnalyzer, an equimolar pool was made of all precleaned and dual-indexed 16S libraries. Sequencing of the library pool was performed on a single instrument run on the Illumina MiSeq platform, using a read length of 2 × 300 bp.

Lipid extraction and quantification

Lipidome analysis was performed from 1 g of powder resulting from the maceration of each coral fragment ($n = 26$, including skeleton) extracted following the BUME method (Löfgren et al. 2016) with modifications as described in (Charriez et al. 2021). A total supernatant volume of 9 mL was homogenized, and 1 mL aliquot of each sample was transferred to LC vial in duplicate for analysis. Lipid samples were analyzed by liquid chromatography followed by high-resolution mass spectrometry (LC-HRMS) in a Thermo Q-Exactive Orbitrap equipped with a heated electrospray ionization (HESI) source. Mass spectrometry and liquid chromatography separation were performed as in Charriez et al. (2021). Lipid identification and relative quantitation were performed using the Lipid Search Software™ (Thermo Scientific, version 4.2.21). Lipid Search parameters were adjusted to include only compounds classified into 8 lipid classes: cholesterol esters (ChE), fatty acids (FA), monoacylglycerols (MG), monogalactosyldiacylgylcerols (MGDG), phosphatidylcholines (PC), phosphatidylethanolamines (PE), triglycerides (TG), and wax esters (WE). These classes were chosen as relevant components of the lipidomes of corals and their symbionts based on previous reports for the case of *A. cervicornis* (Charriez et al. 2021). Data were blank subtracted and filtered to eliminate all peaks with signal-to-noise ratio < 3 and a delta ppm of ± 10. Peak area was normalized using internal standards (Charriez et al. 2021).

DNA methylation library preparation quantification and analysis

Whole genome bisulfite sequencing (WGBS; at Genewiz, South Plainfield, NJ) processing of coral host genomic DNA was performed from "soft" extractions ($n = 26$), using the EZ DNA Methylation-Gold kit (Zymo,CA,USA) and the NEBNext Ultra DNA Library Preparation Kit (New England Biolabs, Ipswich, MA). The resulting libraries were sequenced on a HiSeq platform, generating 150 bp paired end reads. Sequence quality trimming was performed using TrimGalore! (v0.6.4; Martin 2011), removing 10 bp from both the 5′ and 3′ ends. After quality control with FastQC v.0.11.7 (Andrews et al. 2010), sequences were aligned to the *Acropora cervicornis* genome (Dr. I. B. Baums assembly, unpublished) using Bismark (v0.22.3; Krueger and Andrews 2011) with non-directionality and an experimentally optimized alignment score of L,0,-0.9. The Bismark platform was also used to quantify methylated or unmethylated CpGs in all samples. The resulting.cov files were filtered to keep only CpG loci with at least 5 × coverage for each sample to perform the characterization of general methylation patterns. CpG loci were annotated by feature using available annotations for the draft genome. Genes, mRNA, exons, coding

sequences (CDS), flanking untranslated regions (3'-UTR and 5'-UTR) and tRNAs were obtained directly from the genome annotation file, while putative promoter regions, intergenic regions, introns and repetitive regions were created following previously developed pipelines (Venkataraman et al. 2020). Combined methylation calls per CpG locus (i.e., cov files) for all samples were filtered to maintain individual CpG dinucleotides with at least 10× coverage and used to characterize experimental differences in DNA methylation of *A. cervicornis*. Repetitive regions in the genome of *A. cervicornis* were annotated using RepeatMasker v4.1.1 (A.F.A. Smit, R. Hubley & P. Green RepeatMasker at http://repeatmasker.org).

RNA library preparation quantification and analysis

Host-enriched RNA ($n = 26$), normalized to a concentration of 44 ng/uL (30 µL), was shipped to the Genomic Sequencing and Analysis Facility, University of Texas at Austin, for tag-based RNA-seq (tagSeq) library preparation and sequencing on an Illumina HiSeq 2500 (Lohman et al. 2016). Gene expression quantification was performed following code developed by M. Matz (github.com/z0on/tag-based_RNAseq). TagSeq demultiplexed raw reads were trimmed of adaptors and quality filtered using fastx-toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). PCR duplicates were removed, and the resulting reads were mapped to the *A. cervicornis* transcriptome (Libro et al. 2013) using bowtie2 (v.2.3.4; Langmead and Salzberg 2012). Mapping to the draft genome was not successful given the draft status of the annotation and the limitations imposed by only sequencing 3' ends of transcripts as part of the TagSeq protocol. Successfully mapped reads were compiled into a count table for downstream analysis.

Statistical analyses

All statistical analyses were completed in R (v4.0.2; R Core Team 2021) with RStudio (v1.3.959; R Studio Team 2020) unless stated otherwise. Analyses' code is available on a Github repository (see data accessibility statement).

Microbial analyses

SymPortal output, including the absolute abundance of each ITS2 type profile and sequenced reads representative of putative Symbiodiniaceae taxa, were analyzed to evaluate symbiont community divergence between depths using PERMANOVA with the adonis function in the R-package vegan (Oksanen et al. 2019). Fragment identity was used as strata in the models for both ITS2 type profiles and individual taxa distributions, and 9999 permutations of residuals from Bray-Curtis dissimilarities were employed. Using the QIIME2 analysis package, bacterial sequences were denoised using DADA2 with the following parameters: --p-trunc-len-f 280 \ --p-trim-left-f 17 \ -- p-trunc-len-r 220 \ --p-trim-left-r 21. ASVs were classified using the SILVA138 database with the sklearn naïve Bayes classifier. All ASVs with frequency of less than 0.1% of the mean sample depth were removed to avoid contamination. Data statistics, diversity metrics, and ANCOM analyses were calculated using QIIME2.

Global molecular responses

Genome-wide gene expression and DNA methylation were visualized through principal coordinates analysis (PCoA) of Manhattan distances, and differences between sites were tested through ANOVA. Treatment associated variance in DNA methylation and gene expression across all colonies was further analyzed through a Discriminant Analysis of Principal Components (DAPC) using the R package adegenet v2.1.3 (Jombart et al. 2010). Gene expression count data were previously filtered to exclude genes with a mean count <1 and normalized using the variance stabilizing transformation implemented in DESEQ2 (Love et al. 2014). DNA methylation data were previously filtered to include only CpGs with at least 10x coverage and were conserved in all samples. Briefly, Single nucleotide polymorphisms (SNPs) were identified across all samples using BisSNP (Liu et al. 2012). Resulting heterogeneous positions were filtered out, maintaining only conserved CpGs for differential methylation analysis as in (Liew et al. 2020). Methylation was further summarized grouping CpGs with potential differential methylation between all samples (regardless of treatment) at a regional level (100bp windows) using the methylpy DMRfind function (Schultz et al. 2015) (https://github.com/yupenghe/methylpy).

Differential responses of DNA methylation and gene expression

Differentially methylated regions (DMRs) were identified through ANOVA on arcsine-square root-transformed methylation data (previously summarized by DMRfind). Differentially methylated genes (DMG) were identified using a generalized linear model implemented in R, with the model glm(meth, unmeth ~ site, family = binomial). Gene level methylation was summarized across genome regions previously aligned with the available transcriptome (Libro et al. 2013) using NCBI MagicBlast (Boratyn et al. 2019). Regions and genes with p-adjusted values < 0.05 were considered differentially methylated (DMRs or DMGs, respectively). Only CpG positions shared by all samples, with at least 10× coverage, were included in the differential methylation analyses to reduce the potential effect of single nucleotide polymorphisms (see previous section). Differentially

expressed genes were identified using a generalized linear model approach implemented with the R package DESEQ2 with the formula ~genotype + site. Genes with FDR ≤ 0.1 were considered differentially expressed.

Functional enrichment analysis

Analyses of gene ontology (GO) and eukaryotic orthologous groups (KOG) enrichments associated with gene expression and methylation were performed using GO-MWU (Wright et al. 2015) and KOG-MWU (Matz 2016), respectively. These were based on log2 fold of the methylated/unmethylated fraction of all CpG loci per gene, in the case of gene-level DNA methylation, and on signed log(*p*-values) for gene expression. *A. cervicornis* KOG and GO categories were obtained from a previously published transcriptome for the species (Libro et al. 2013).

Results

Coral demographic and physiological responses to environmental differences between outplanting sites

Differential survival of outplants

The survival rate among the 400 outplanted coral fragments was 41.3% after one year. Survival was highly dependent on the particular reef where these fragments were outplanted ($p < 0.0001$) and the depth ($p = 0.00056$). Coral genotype also showed a significant effect over survival ($p = 0.012$), but with a lesser importance than reef and depth in a Random Survival Forest model. LP corals survived better than CR (Fig. S1B), and the deep sites caused higher mortality than the shallow (Fig. S1C). The 12 genets included in the physiological analyses showed no significant differences in performance between them (Fig. S2B), but as a group showed significantly better survival probabilities than all other genets (Fig. S1D; $p = 0.0042$). Differences between outplanting depth persist when only these colonies are analyzed (Fig. S2A). Overall, fragment survival was influenced by outplanting site (reef and depth) and coral genotype, although no differences were observed between the genets selected for the physiological and molecular analyses. Deep reefs imposed challenging environmental conditions to outplants, resulting in higher mortalities across all reefs and genets.

Microbial community composition

The sequencing and analysis (SymPortal) of the ITS2 region identified 69 symbiont sequence variants, collapsed into 6 profiles across all analyzed samples ($n = 26$, Fig. 1A). ANOVA analyses found a significant effect of colony identity on the beta diversity of ITS type profiles ($F = 1.0259e31$, $df = 12$, p-value $< 2.2e{-}16$), but no differences between outplanting sites ($F = 0.2188$, $df = 1$, p-value $= 0.6442$). Similarly, no effect of outplanting depth was found for ITS2 sequence distribution (PERMANOVA, $R^2 = 0.00535$, $df = 1$, p-value $= 0.7703$). The microbiome sequencing produced a mean of 125,314 denoised reads per sample (range: 77,490–194,167). Only features represented by a minimum of 125 denoised reads were included in the analysis,

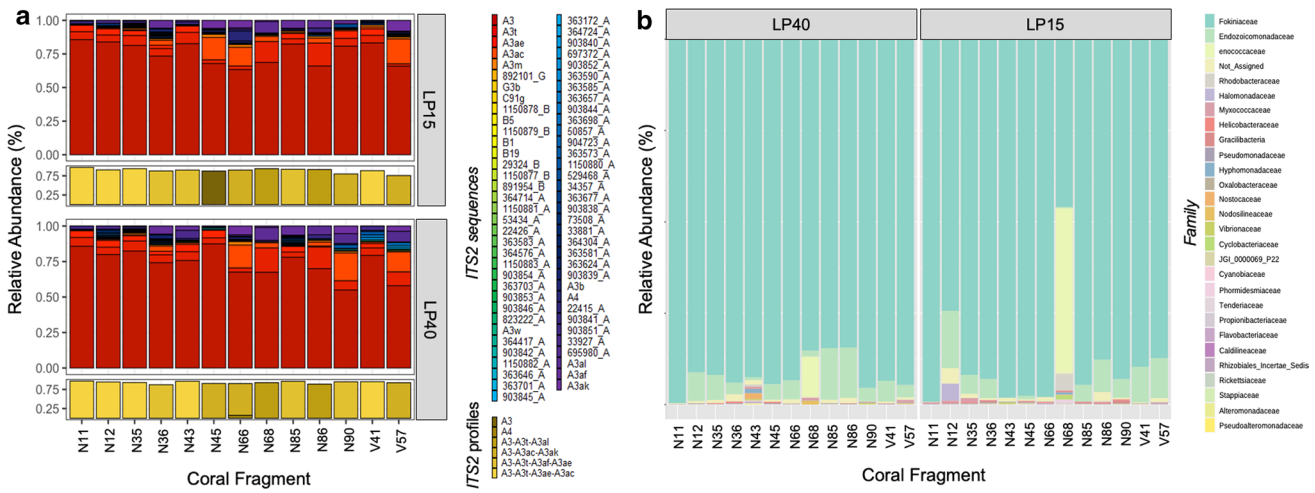

Fig. 1 Microbial community divergence. **A** Relative abundance of ITS2 sequences and predicted profiles of symbiont types; **B** Relative abundance of bacterial clades for *A. cervicornis* ramets outplanted to 3 m (LP15) and 15 m (LP40) depths, and sampled one-year post-outplanting. Only ITS2 sequences contributing > 0.01% in at least 1 sample are labeled. Named sequences (e.g., A3ae or A4) refer to sequences used to characterize the ITS2 type profiles, or sequences commonly found in previous analysis through SymPortal (Hume et al. 2019). Other lesser representative sequences are shown with their database ID and their clade identifier (e.g., 29324_B)

to mitigate contamination from bleed-through. Amplicon Sequence Variants (ASVs) corresponding to members of 21 bacterial orders and 27 families were taxonomically identified. No significant differences were observed in the microbial community between the two depths (Fig. 1B and S1; PERMANOVA: *F*-value: 0.58976; *R*-squared: 0.023984; *p* value = 0.788). ANCOM analysis also identified no significant features diverging between the two depth treatments. Overall, the microbial community seems to remain stable regardless of the marked differences of the environmental conditions of the outplanting sites.

Lipidome composition

A total of 3062 lipidic compounds were identified across all samples within the eight lipid classes targeted as relevant for coral lipidomes (Charriez et al. 2021). Among these, monogalactosyldiacylgylcerols (MGDG) presented the highest number of compounds (217.5 compounds per sample, Fig. 2A). However, the most abundant (mass proportion) lipid classes were triglycerides (TG) followed by monoacylglycerols (MG) (Fig. 2B). A significant effect of the outplanting site and its interaction with the lipid class in the number of compounds found was observed (Table S2), with significant changes in classes TG (Paired *t*-test: $t = -3.9567$, $df = 12$, p-value = 0.002) and wax esters (WE; Paired *t*-test: $t = -2.5695$, $df = 12$, p-value = 0.0246), and marginally significant in MGDG (Paired *t*-test: $t = -2.1071$, $df = 12$, p-value = 0.0568) and phosphatidylcholines (PC; Paired *t*-test: $t = -2.0694$, $df = 12$, p-value = 0.0608; Fig. 2A). Similarly, the relative abundances of lipids within each class were dependent on the coral colony (PERMANOVA: $R^2 = 0.59617$, $df = 12$, p-value = 0.0029; Fig. 2B). However, for this variable, all classes were dependent on the outplanting site (Contingency tests, Table S3), with the most dramatic changes occurring in phosphatidylethanolamines (PE; reduced in half on the deep site) and MGDG (doubled in the deep site) (Fig. 2B). Overall, these results support a divergence in coral lipidic composition influenced by depth.

Coral molecular responses to environmental variation between outplanting sites

Gene expression changes

RNA sequencing (Tag-Seq, $n = 26$ samples) produced a total of 309,224,452 reads, resulting in 45,518 transcripts utilized for downstream analyses after trimming and quality filtering (Table S4, Fig. S4). Significant inter-genet variability was evidenced through PCoA analysis of variance-stabilized gene counts (Fig. 3A), with samples clustering primarily by colony (Fig. S5). However, consistent divergence between ramets related to the outplanting site can be observed across PC1 (explaining 12.6% of the total variance), which is further corroborated by DAPC (Fig. 3B). Given the high variability observed in the PCoA analysis, differentially expressed genes (DEGs) between corals outplanted to each depth were identified across all genotypes. 306 DEGs were identified across all genets, with up-regulated genes in the deep reef almost doubling those up in the shallow (109 up in shallow, 197 down in shallow).

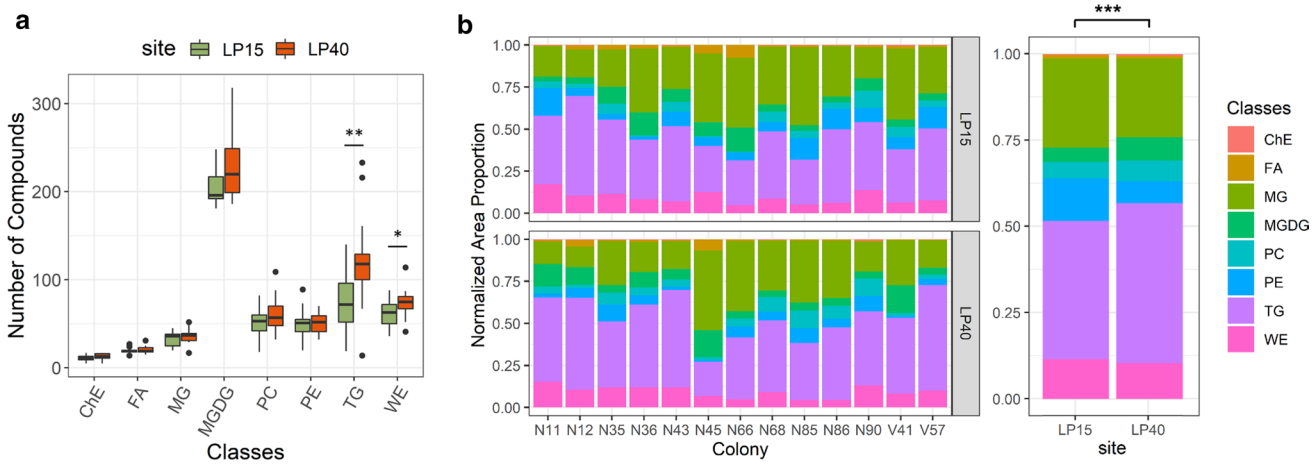

Fig. 2 Lipid profiles derived from coral ramets maintained at 3 m (LP15) or 15 m (LP40) for a year. **A** Lipid extraction results expressed as the number of compounds per each of eight lipid classes. Results of pairwise comparisons through t-test between sites are represented by asterisks: (*)$p < 0.05$; (**)$p < 0.01$. **B** Proportional abundance of lipids (calculated from normalized peak areas) per class across all genets and outplanting sites. Asterisks represent significance of chi-square tests for each class ($p < 0.001$). ChE cholesterol ester, FA Fatty acid, MG mono-acyl glycerol, MGDG Monogalactosyldiacylgylcerol, PC Phosphatidylcholine, PE Phosphatidylethanolamine, TG Triglyceride and WE Wax ester

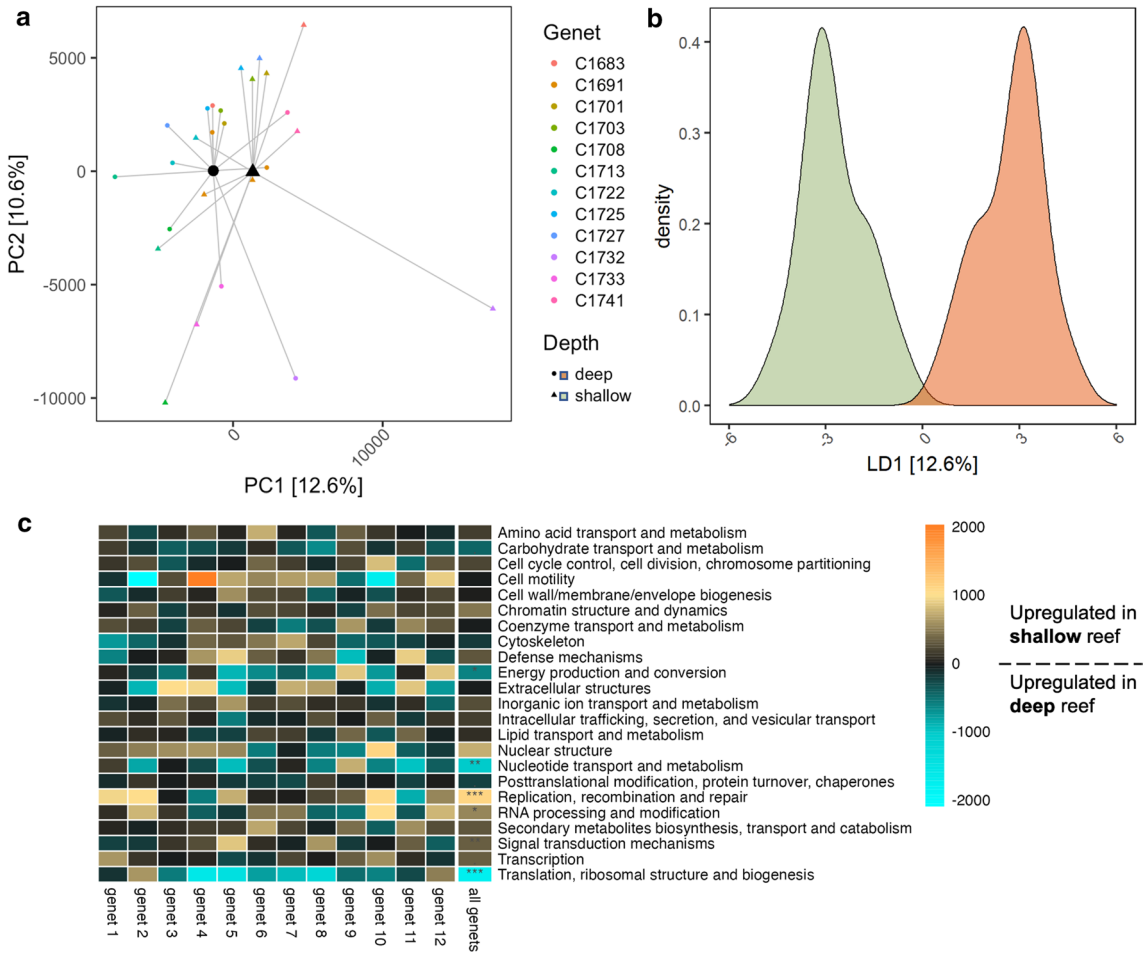

Fig. 3 Divergence of transcriptional state in *A. cervicornis* ramets maintained at 3 m or 15 m for a year. **A** Principal Coordinate Analysis (PCoA) of "manhattan" distances based on variance-stabilized gene counts. Larger symbols represent the centroid of the sample distribution for each depth. **B** Discriminant analysis of principal components (DAPC) **C** Eukaryotic ortholog group (KOG) enrichment analysis results. KOG functional categories up- or down-regulated (indicated by color) for the contrast, *shallow vs deep reef*, of each individual genet and the combination of all genets. Statistical significance for all genets is represented as: (.) FDR < 10%; (*) FDR < 5%; (**) FDR < 1%; (***) FDR < 0.1%

GO (Table S5) and KOG (Fig. 3C, Table S6) functional analyses, revealed significant functional responses across all genotypes, regardless of the substantial variability between sampled genets. Corals outplanted to the shallow reef (LP15) significantly up-regulated KOG categories including "replication, recombination and repair," "RNA processing and modification," "signal transduction mechanisms," and "transcription," while corals maintained in the deep reef (LP40) up-regulated genes involved in "translation, ribosomal structure and biogenesis," "nucleotide transport and metabolism," "energy production and conversion," "carbohydrate transport and metabolism" and "post-translational modification, protein turnover, chaperones" (Fig. 3C, Table S6). Similar functional categories were also evidenced in the GO analysis (Table S5), with corals outplanted to the deep site upregulating mostly components and functions of the cellular energetic metabolism, and corals in the shallow reef up-regulating mostly RNA processing and signal transduction elements and functions. Overall, gene expression analyses are consistent with a divergence in transcriptional profiles, suggesting the presence of differences in energetic states and stress response influenced by local environmental conditions between outplanting sites.

DNA methylation landscape

Sequencing of WGBS libraries ($n = 26$) produced a median of 40,706,732 reads after trimming and quality filtering. Sequencing and mapping statistics were homogeneous (low inter-sample variation), reducing potential biases for comparisons between treatments (Fig. S6). After filtration, 17,428,204 CpGs (91% of all CpGs in the *A. cervicornis* genome) were used for methylome characterization. Most of the CpGs in the *A. cervicornis* genome (82.9%) were

unmethylated. On the other hand, 1,981,493 CpGs (11.4%) displayed methylation levels over 50%, and only 5.7% showed sparse intermediate methylation (methylated reads between 10 and 50%). Methylated Cytosines were prevalent in gene bodies (as expected in invertebrates) displaying significantly higher % methylation levels when compared with intergenic regions (Fig. S7A, B). While introns showed significantly higher methylation levels than exons (Fig. S7B), methylated CpGs were more frequent at gene boundaries, including the first and last exons, and flanking regions (Fig. S7C).

A total of 146,849 methylation islands were identified in the genome of *A. cervicornis*, containing between 11 and 13,212 CpGs (mean = 42 CpGs), a length ranging between 500 a 395,513 bp (mean = 1,576 bp), and predominantly overlapping with gene bodies (50,751, 34.56% with exons; 48,494, 33.02% with introns). Interestingly, the proportion of methylation islands overlapping with genomic features was significantly different when compared with the general distribution of CpGs in the genome (Contingency test; p-value < 0.001, Fig. S8A), with a 3 × enrichment in exons and a 26 × enrichment in repetitive regions (transposable elements). Remarkably, repetitive regions in the genome of *A. cervicornis* account only for approximately 1.4% of the genome [less than half of the regions in the genome of *Montastraea cavernosa* (Rodriguez-Casariego et al. 2022)], with only 145 retroelements and 45 DNA transposons.

Divergence of DNA methylation patterns

Statistically significant differentially methylated regions (DMRs) were evaluated for genomic windows (100 bp length) across all samples regardless of their treatment. As observed for gene expression analyses and regardless of an exhaustive filtering of SNPs, DNA methylation patterns were highly influenced by coral genotype, as evidenced by PCoA and clustering analyses (Fig. 4A, S9). However, outplanting site effects were less clear in this case. DAPC analysis (Fig. 4B) showed divergence of the methylation patterns only on a subset of samples along LD1, explaining 31.5% of the total variance. ANOVA analyses were performed to further evaluate outplanting site effects, identifying 130 DMRs (p.adj < 0.05) across all genets between the two outplanting sites. A substantial variability across genets was also evidenced in the methylation level of DMRs (Fig. 4C). A significant portion of these DMRs occur in coding regions

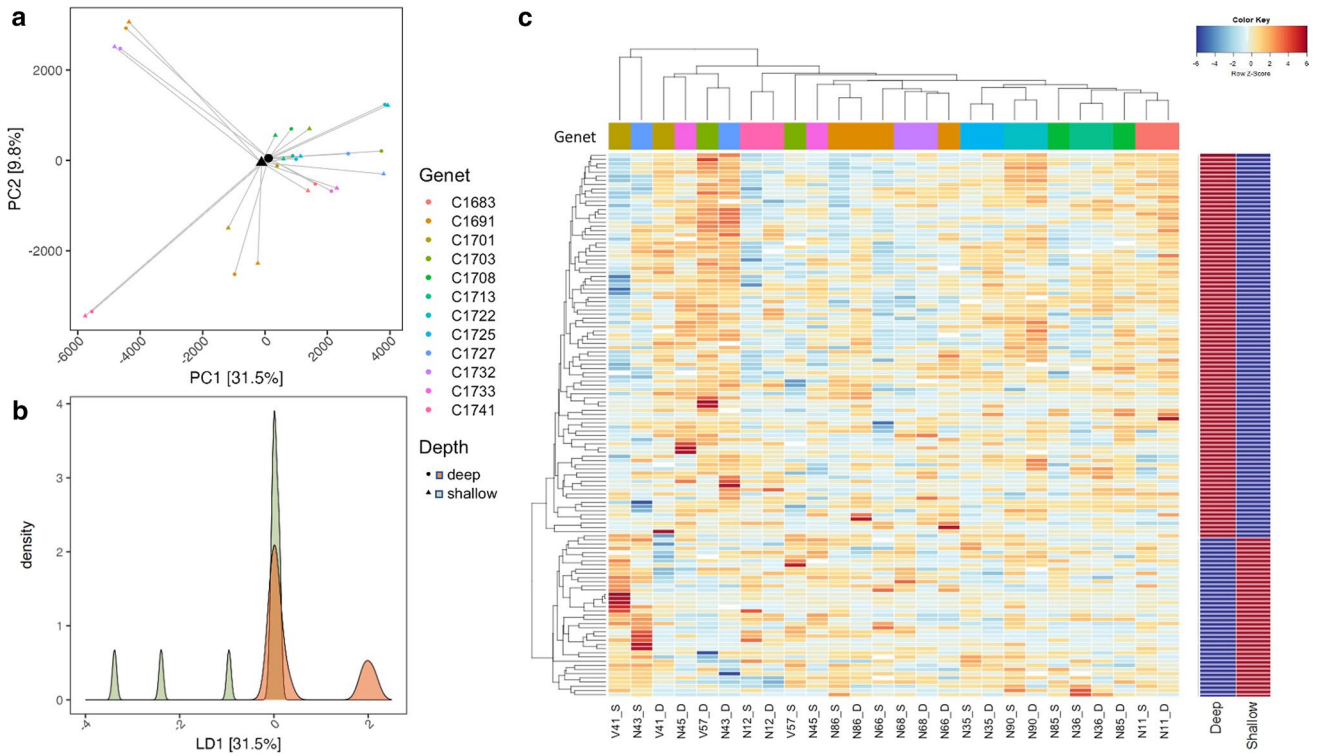

Fig. 4 General DNA methylation patterns in *A. cervicornis* ramets maintained at 3 m or 15 m for a year. **A** Plot of the first two principal components from a principal coordinate analysis (PCoA) by depth and genotype, based on "manhattan" distances of percent methylation of 100 bp windows. **B** Density plot of a discriminant analysis of principal components. **C** Heatmap illustrates methylation level variability across 130 differentially methylated regions (DMRs) between all samples (left) and grouped by outplanting sites (right). NXX or VXX refer to initial colony IDs; _S (shallow reef) and _D (deep reef). Genotype correspondence is color coded under column clustering

(CDS) and transposable elements (Fig. S8B), with a significant reduction of the proportion of DMRs present in intergenic regions.

Given the observed prevalence of differential region DNA methylation in gene bodies (CDS), the presence of differentially methylated genes (DMGs) was evaluated through ANOVA on a set of 32,535 genes containing at least 5 conserved CpG positions covered ≥ 10× in all samples (very conservative filtering to ensure relevant variability across genets). A total of 1,798 DMGs were identified between sites across all genets (Fig. 5A). Only 4 genes were both differentially methylated and expressed (Fig. S10D), but a significant correlation of gene body DNA methylation with gene expression (positive; Fig. 5C), and with the coefficient of variation of gene expression (negative; Fig. 5D) was observed. However, no correlation between differential methylation and gene expression, or differential expression and methylation level was observed (Fig. S10A–C), not supporting a possible interaction between treatment-induced DNA methylation changes and gene expression. In terms of gene function, both KOG-MWU and GO-MWU analyses did not find any term significantly (FDR < 0.05) over- or under-methylated across all genotypes.

In summary, gene body DNA methylation displayed statistical divergence in a subset of genes across sites, with general patterns showing a strong influence of the genet identity. However, a clear link between site-induced changes in DNA methylation and subsequent modifications in gene expression was not observed.

Discussion

Stability of coral symbiotic and bacterial communities

The present work found that stability, rather than plasticity, best describes the Symbioninaceae community (Fig. 1A) and bacterial assemblages (Fig. 1B) in the studied coral

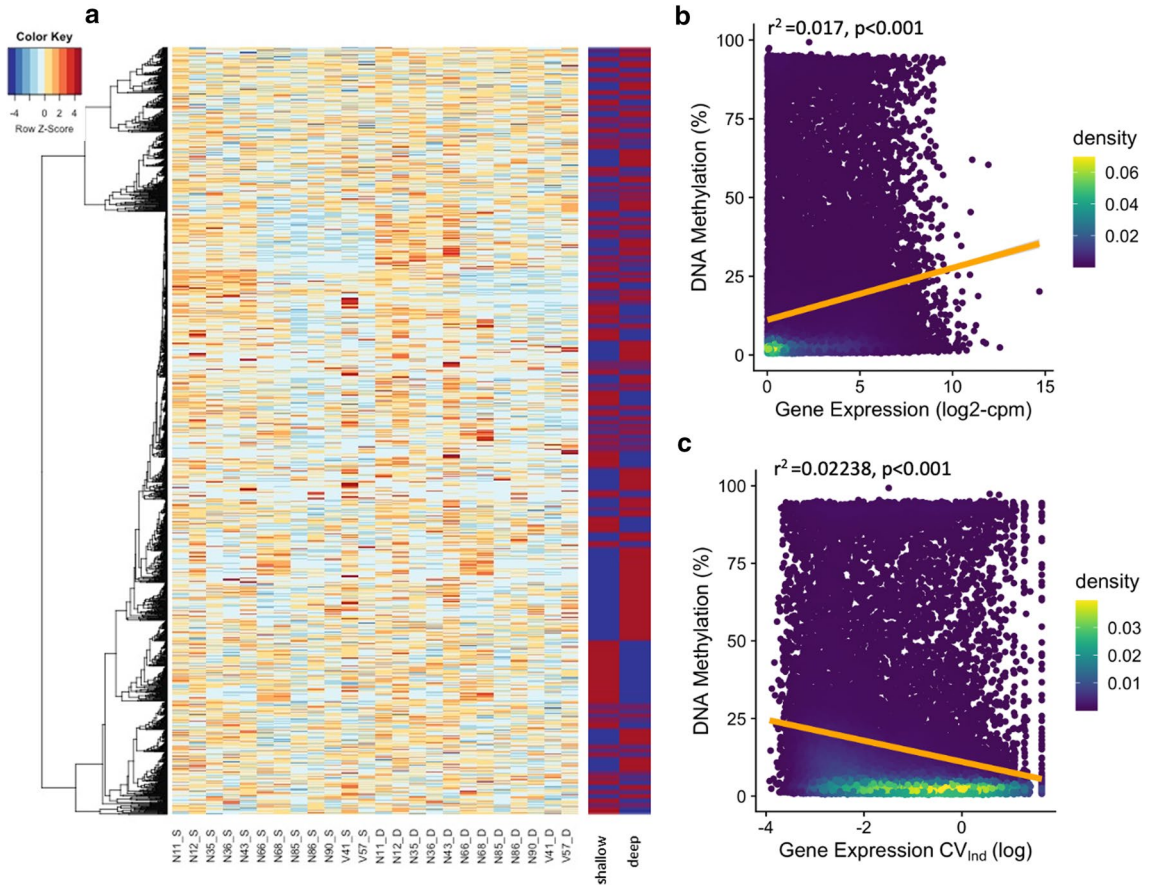

Fig. 5 Divergence of gene body methylation in *A. cervicornis* ramets maintained at 3 m or 15 m for a year. **A** Heatmap of methylation level for 742 differentially methylated genes (DMGs) by sample (left) and grouped by site (right). NXX or VXX refer to initial colony IDs; _S (shallow reef) and _D (deep reef).). **B, C** Density plots of gene level DNA methylation correlation with gene expression level **(B)** or its coefficient of variation **(C)**. Equations represent linear models of mean gene DNA methylation as predictor and either mean gene expression or coefficient of variation as response

fragments under different environmental conditions. Although the genus *Acropora* is known to host a highly diverse symbiotic community (Putnam et al. 2012; Williams et al. 2022; Miller et al. 2020), a remarkable stability of the consortia has been observed in studies monitoring seasonal variation (Rodríguez-Casariego et al. 2020; Thornhill et al. 2006) as well as in experimental manipulations (O'Donnell et al. 2018; Indergard et al. 2022), including transplantation (Aguirre et al. 2022), with mixed and dose-dependent results observed under stress (Singh et al. 2023; Thornhill et al. 2006; Klinges et al. 2023). Maintaining such stability under divergent environmental conditions indicate either a limited role of the symbiotic composition modulating phenotypic plasticity in this context, a remarkable plasticity of the symbiome to maintain appropriate interactions under such divergent conditions without affecting the community structure, or a very strong match between the host and the symbiotic community that persist at the expense of other mechanisms showing plastic responses. However, the role of background symbiotic species (representing < 0.1% of the community) modulating holobiont's phenotypic plasticity has been highlighted before (Jung et al. 2021; Lewis et al. 2019). Therefore, imperceptible changes not influencing statistical significance could have a relevant biological impact.

Divergence of lipidic profiles indicate acclimatization to fulfill increased energetic demands in deep-outplanted corals

Consistent changes in both diversity and abundance of lipidic compounds were observed between depths, with wax esters (WE) abundance proportionally higher in the shallow reef site and triglycerides (TG) more abundant in the deep reef site (Fig. 2). Reductions in the abundance of WE have been linked to stress (Grottoli et al. 2004; Yamashiro et al. 2001), and "frontloading" of both WE and TG lipid classes have been shown to reduce thermal stress-related mortality (Anthony et al. 2007). As the main component on eggs (Arai et al. 1993), a depletion of WE in deeper sites could be indicative of depth-induced reduction in reproductive potential (Liberman et al. 2022). Additional reductions in monoacylglycerols (MG) and phosphatidylethanolamines (PE) in the deep reef (Fig. 2B), could be indicative of a response to stress (Grottoli et al. 2004; Solomon et al. 2019), consistent with our survival results, but also of a lower energetic budget given the lower irradiance of this site, and/or the demand of calcification under lower pH.

The high diversity of monogalactosyldiacylgylcerols (MGDG) (Fig. 2A) can be directly linked with the high symbiont diversity characteristic of this species, given that lipids of this family constitute one of the main components of thylakoid membranes (Guschina and Harwood 2009). Consistently, significant increases in MGDG lipids at deep reef conditions (Fig. 2B) could suggest a plastic response to stress (Rosset et al. 2019) through the strengthening of thylakoid membranes in the symbionts, or an increase in symbiont population to compensate for lower carbon translocation under lower irradiance (Tremblay et al. 2014). However, while the observed increase in lipidic diversity in the deep-reef is difficult to accommodate in the two previous hypotheses, given the observed stability of the symbiont community, the activation of heterotrophy in response to the lower pH of the deep site (Edmunds 2011; Towle et al. 2015) could explain both changes observed in the abundance and diversity of MGDG class lipids. Similarly, TG and WE classes presented a significantly higher number of compounds for corals outplanted to the deep site, adding to the possibility of this being a result of additional dietary items obtained through heterotrophic feeding. Overall, the analysis of the lipidome provides evidence of both plastic responses to stressful conditions on the deep site, and shifts to accommodate new energetic demands, potentially modulated by a shift to a more heterotrophic diet, supporting energetic demands and reducing physiological stress in surviving colonies.

Transcriptional responses suggest favorable acclimatization to the conditions in the deep reef

The obtained results revealed the presence of high intergenet variability (Fig. 3A, S5), supporting previous reports describing similar observations for gene expression (Cunning and Baker 2020; Parkinson et al. 2018; Granados-Cifuentes et al. 2013). Therefore, only common divergent transcriptional profiles were described in response to transplantation to different environments (Fig. 3B). The largest site-specific effect was the differential regulation of ribosomal biosynthesis, transcription, translation, and energetic metabolism (Fig. 3C). This response was evident in both the KOG and GO enrichment analyses (Fig. 3C, Supplementary Table S5 and S6), and was consistent across genets, with 8 out of the 12 genets in the study up-regulating in the deep reef "translation, ribosomal structure and biogenesis." Since the increase in these categories is often related with cellular proliferation (López-Maury et al. 2008) and growth, this result would be indicative of a more favorable energetic condition in corals of the deep reef. This is further supported by the link between ribosomal synthesis and energetic metabolism (Grummt and Ladurner 2008) as hinted by the lipidome, and by the link between protein synthesis and the activation of mechanisms counteracting lower carbon translocation, such as for example by hosting larger symbiotic populations (Cunning and Baker 2020), or by activating heterotrophic nutrition in response to a higher energetic demand. Given the lack of data describing symbiont abundance we cannot conclude in favor of one or other

mechanisms. However, based on the observed lipidic profiles, it's probable that either the lower pH in the deep reef and/or the limiting conditions for autotrophic functioning could have promoted a shift to heterotrophy (Towle et al. 2015), accounting for the up-regulation of the energetic metabolism evident in the transcriptional response. Contrary to the metabolic depression documented in other marine invertebrates (Strader et al. 2020), several studies have also found increases in coral energetic metabolism under ocean acidification (Davies et al. 2016; Herrera et al. 2021; Kenkel et al. 2018; Ogawa et al. 2013; Vidal-Dupiol et al. 2013), with a similar up-regulation of ribonuclear protein biosynthesis and energetic metabolism (Herrera et al. 2021; Kenkel et al. 2018).

Corals in the shallow reef upregulated transcripts involved in repair mechanisms and signal transduction, and downregulated those involved in ribosomal and energetic metabolism (Supplementary Tables S4 and S5), all representing hallmarks of corals environmental stress response (López-Maury et al. 2008). Such behavior is consistent with the higher thermal variability and light irradiance in the shallow reefs (Table S1), in agreement with the transcriptional responses observed in *Porites astreoides* transplanted between inshore and offshore reefs (Kenkel and Matz 2016). Thus, the remarkable similarities between the results of the present study and other studies focused on ocean acidification could indicate that differences in pH between the outplanting sites (Table S1; Rodríguez-Casariego et al. 2020) are responsible for driving part of the transcriptional divergence observed. However, given the direct comparison between experimental groups (no controls), up-regulation of a transcript in an experimental group could be in fact the results of its downregulation in the other. Accordingly, a combination of differential responses to stress in the shallow reef (likely irradiance and thermal conditions), and deep reef (pH conditions) account for both the divergence observed in lipidome and transcriptome variation. This transcriptional response in the deep reef should not be taken as a signal of enhanced conditions for coral development, representing a potential refuge from stress. Our survival results confirm that the deep reef imposes conditions that result in higher mortality, and only through substantial phenotypic plasticity some colonies are able to survive.

DNA methylation divergence is present at genome-wide regional and gene-specific levels

A single-base resolution methylome of *A. cervicornis* is described for the first time in the present work using WGBS. With a high read depth and mapping efficiency (Supplementary Fig. S6), over 90% of all the CpG sites in the genome of the species were appropriately characterized. The observed DNA methylation patterns were dependent on the coral genotype, as previously described (Dimond and Roberts 2020; Liew et al. 2020; Rodríguez-Casariego et al. 2020), with most samples grouping by genet in a multidimensional space (Fig. 4A). Regardless of this variability, significant differences at regional, and gene-specific levels were observed (Fig. 4B, C and 5A), with some increases in the level of gene body methylation in corals outplanted to the deep reef. Similarly to the lipidome and transcriptome responses, the consistently lower pH levels occurring in the deep reef during the experiment (Table S1; Rodríguez-Casariego et al. 2020) could potentially influence the methylation state of the fragments exposed to these conditions, as increased global and gene body DNA methylation have been observed in corals exposed to low pH (Liew et al. 2018; Putnam et al. 2016). Alternatively, an energetically favorable acclimatization to the environment of the deep reef, as a result of a shift to heterotrophy, would be conducive to higher methyltransferase activities (Donohoe and Bultman 2012) reducing spurious transcription (Li et al. 2018), and therefore allowing cellular proliferation as evidenced in the transcriptional profile of deep reef corals.

Regardless of the commonalities between phenotypic changes and methylome responses observed in the present work (Fig. 6C, D) and in previous studies in other cnidarians (Dixon et al. 2018; Dixon and Matz 2022; Li et al. 2018), solid evidence of the regulatory role of inducible DNA methylation mediating changes in coral gene expression is still elusive (Fig. S10, Dixon and Matz 2022). It has been suggested that an indirect interaction between DNA methylation and gene expression could occur through the modulation of regulatory regions far from the target gene [trans regulatory elements (Shi et al. 2012)], or through crosstalk and cooperation with other epigenetic mechanisms in a context-dependent manner (Nawaz et al. 2022). For instance, a correlation between DNA methylation and gene expression has been recently established within the context of an open chromatin state during the development of a sea urchin species (Bogan et al. 2023). Similarly, in the cnidarian model Aiptasia the co-occurrence of post-translational modification of H3-histones and DNA methylation seem to cooperate in promoting gene expression. On the other hand, inducible methylation in corals could be linked only with the hypothesized ancestral role of silencing transposable elements (Bestor 1990). Proportional enrichments of methylated CpG's and differential methylation in transposable elements have been observed in several coral species (Rodriguez-Casariego et al. 2022; Ying et al. 2022). Yet, the evolution of DNA methylation in cnidarians appears to be complex (Zhang and Jacobs 2022), as suggested by the inconsistent DNA methylation patterns observed across and within taxa. Therefore, further studies will be required to fully elucidate the role/roles of DNA methylation in corals,

including the expansion of multi-mechanism interaction analyses and genome-wide associations.

Conclusions

The different -omic analyses developed in the present work support the rapid phenotypic responses of *A. cervicornis* to the heterogeneous environmental conditions imposed by the different outplanting sites. Remarkably, the stability observed in the symbiotic community raises the possibility that this coral species relies, and perhaps importantly, on host regulated mechanisms to rapidly acclimate to divergent environmental conditions, potentially including nutritional plasticity. This is further supported by previous reports indicating that corals modulate their feeding modes under different environments, providing a potential adaptive mechanism to sustain growth and avoid mortality under stressful conditions (Anthony and Fabricius 2000; Ferrier-Pagès et al. 2011; Tremblay et al. 2014, 2016). Both the lipidomic and transcriptional responses observed in the present work support this notion, suggesting a transition toward more heterotrophic nutrition in corals subject to lower pH at higher depth. Although DNA methylation changes are also in agreement with a response to elevated pH, conclusive evidence for the direct regulatory role of DNA methylation on gene expression changes is still missing. Focusing on the interaction among epigenetic mechanisms, and in the interconnectivity of energetic metabolism and epigenetic regulation in the modulation of phenotypic plasticity, could improve our mechanistic understanding of rapid adaptive responses and gene regulation in corals.

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Data accessibility Raw sequence data are available at the NCBI Sequence Read Archive under accession number PRJNA938148. Pipelines details, code and processed datasets can be found at https://github.com/jarcasariego/ACER_clonal_divergence and archived at Zenodo https://doi.org/10.5281/zenodo.7661386.

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