



Comprehensive Metatranscriptome Analysis of the Reef-Building Holobiont *Millepora complanata*

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INTRODUCTION

Hydrocorals from the genus *Millepora* (Phylum Cnidaria, Class Hydrozoa) are conspicuous organisms commonly found in shallow waters of tropical regions. These cnidarians, which are considered the second reef builders in the world (Rojas-Molina et al., 2012), are vital components of marine ecosystems and provide habitat for many coral reef-dependent communities (Lewis, 2006).

The genus *Millepora* includes calcareous hydroids that are not classified in the group of true stony corals (Radwan and Aboul-Dahab, 2004). Milleporids are commonly known as “fire corals” due to their ability to inflict painful wounds in human epithelial tissues through the secretion of toxins (Radwan, 2002), which are stored in the nematocysts (Shiomi et al., 1989; David et al., 2008).

Millepora complanata is a common resident of the Mesoamerican Reef System in the Mexican Caribbean (Ibarra-Alvarado et al., 2007). This organism inhabits shallow water and the reef crest (Stromgren, 1976) where it forms plate-like colonies composed of multiple clones (Lewis, 1991). Like many scleractinian corals and other cnidarians, *M. complanata* lives in mutualistic symbiosis with dinoflagellate algae of the family Symbiodiniaceae (commonly known as zooxanthellae) (Davy et al., 2012).

Dinoflagellates reside in the symbiosomes, which are phagosome-derived vacuoles within cnidarian gastrodermal cells (Lehnert et al., 2014). Symbiodiniaceae algae provide photosynthetic fixed carbon to their host, in exchange, cnidarians supply their symbionts with nitrogenous compounds (Sproles et al., 2018). Within host tissues, coral-associated symbionts may reach population densities of several millions per square centimeter and the holobiont may contain more than one Symbiodiniaceae genus (LaJeunesse, 2002). Diverse studies have analyzed the symbiotic relationship between stony corals (class Anthozoa) and their symbionts (Furla et al., 2005; Davy et al., 2012; Gusmão et al., 2020; Tivey et al., 2020; Xiang et al., 2020). However, hydrocoral-Symbiodiniaceae symbiosis is poorly understood. Investigations on the mutualistic relationship between reef-building cnidarians and Symbiodiniaceae algae have increased in the “omics age” (Meyer and Weis, 2012; Bi et al., 2019; Simona, 2019) and the sea anemone *Exaiptasia diaphana*, formerly known as *Aiptasia pallida*, has been widely employed as a model to study cnidarian endosymbiosis (Lehnert et al., 2012; Tortorelli et al., 2020). A gene expression analysis of cnidarian-algae association in *Anthopleura elegantissima* evidenced a complex modulation of host gene expression related to oxidative stress, apoptosis, cell proliferation, cell adhesion, and lipid metabolism (Rodríguez-Lanetty et al., 2006).

It has been demonstrated that reef-building cnidarians are also associated with a diverse community of bacteria and other microorganisms (Tandon et al., 2020). These microorganisms are key components of the coral holobiont and play a major role in nutrient cycling, which is fundamental for reef development (Rajasabapathy et al., 2020).

Environmental stressors, such as UV radiation and high temperature can lead to “bleaching,” the disruption of the symbiosis between cnidarians and Symbiodiniaceae (Hughes et al., 2017). Unfortunately, the frequency and seriousness with which bleaching occurs have increased in recent years, seriously compromising coral populations around the world (Sully et al., 2019). Considering that perturbations induced by climate change have threatened the integrity and resilience of marine ecosystems, several investigations have analyzed the bleaching phenomenon, using transcriptomic approaches to understand the molecular mechanisms underlying the responses of cnidarians and symbionts to heat stress (Mayfield et al., 2011, 2014; Pinzón et al., 2015).

Investigations aimed at characterizing the transcriptomes of reef-building cnidarians have focused on species of the class Anthozoa (Kitchen et al., 2015; Kenkel and Bay, 2017; Pootakham et al., 2018; Veglia et al., 2018; Rivera-García et al., 2019; Ryu et al., 2019; Chiu et al., 2020). Regarding organisms of the class Hydrozoa, analysis of the transcriptomes from *Hydractinia echinata* and *Hydra vulgaris* showed the presence of evolutionary-conserved proteins and hitherto undescribed sequences in metazoans (Soza-Ried et al., 2010; Wenger and Galliot, 2013). However, with respect to *de novo* transcriptome assemblies of cnidarians of the genus *Millepora*, only one study has been reported on the branching hydrocoral *M. alcicornis* from the Caribbean (Ortiz-González et al., 2017).

RNA-seq-based transcriptomic approaches have become powerful tools directed toward recognizing all the expressed genes codified by a genome under certain conditions. Additionally, generation of new gene catalogs enables identification of ortholog sequences across related taxa. In this study, we used a metatranscriptomic sequencing approach to describe, for first time, the metatranscriptome of *M. complanata* and its associate symbionts in order to generate a new reference metatranscriptome of reef-building cnidarians of the class Hydrozoa.

DATA DESCRIPTION

Sample Collection

Fragments of *M. complanata* colonies (~10 cm) were collected by SCUBA (Self-Contained Underwater Breathing Apparatus) diving at the Parque Nacional Arrecife located in Puerto Morelos, Quintana Roo, México (21°00′00″ and 20°48′33″ North latitude and 86°53′14.40″ and 86°46′38.94″ West longitude), during November 2016 (Permit No PFP/DGOPA-139/15). Samples were immediately frozen in liquid nitrogen and transported to the “Laboratorio de Investigación Química y Farmacológica de Productos Naturales in the Universidad Autónoma de Querétaro,” Querétaro, Mexico for RNA isolation.

RNA Extraction

Total RNA from three biologically independent *M. complanata* colonies was extracted according to Valderrama-Cháirez et al. (2002) with some modifications. Briefly, 0.1 g of hydrocoral tissue (previously ground with mortar and pestle in liquid nitrogen) was mixed with 1 mL of extraction buffer (150 mM Tris base, 2% SDS, 1% 2-mercaptoethanol, and 100 mM EDTA adjusted to pH 7.5 with saturated boric acid). Afterward, 100 μ L of potassium acetate 5 M, 250 μ L of absolute ethanol, and 500 μ L of chloroform-isoamyl alcohol (25:1) were added and mixed by vortex for 1 min. Samples were centrifuged at 12,000 rpm for 10 min, the top layer was removed and 500 μ L of lithium chloride 8 M were added. Samples were stored overnight. Extracted RNA was precipitated by centrifugation at 12,000 rpm for 10 min, washed with 250 μ L of ethanol 70% and re-suspended in sterile water. Isolated RNA was resolved with a 4% agarose electrophoresis gel (run conditions: 75 V during 60 min) and the RNA integrity number was measured with an Agilent™ Eukaryote Total RNA Nano chip. Run results generated by the Nexseq500 sequencing system yielded 39.18 Gbp with high quality reads (78.43% \geq Q30) in 2 \times 150 medium performance plate.

cDNA Libraries Preparation, Sequencing, and Metatranscriptome Assembly

RNA samples were sent to the “Laboratorio de Servicios Genómicos,” LANGEBIO CINVESTAV-Campus Irapuato for cDNA libraries preparation with Truseq RNA sample prep v2 of Illumina, according to the manufacturer’s instructions. Subsequently, cDNA libraries were sequenced on the Illumina NextSeq500™ platform (2x150) generating 35 GB and 120 million reads. Quality control of raw reads was assessed using FASTQC (Brown et al., 2017), none of the libraries showed any issue. Each library was independently assembled with rnaSPAdes (Bankevich et al., 2012). To improve the accuracy of isoform reconstruction after initial assembly, all sample contigs were merged to reduce sequence redundancy using CD-HIT-EST (Li and Godzik, 2006). A unique identifier was assigned to each FASTA sequence of the assembled transcriptome. Raw data obtained from the sequencing was submitted to the NCBI Sequence Read Archive (SRA) database (Bioproject accession: PRJNA524427; Biosample accession: SAMN11026413). Pair-end reads yield displayed as a maximum 16,431,435 reads. FASTQC analysis of all samples showed a GC content of 40.3%. Assembled transcripts resulted in an N50 value of 510 and an average contig length of 480, whereas statistics of transcriptome assemblies previously obtained from *M. alcicornis* showed an N50 value of 749 and an average contig length of 550 (Ortiz-González et al., 2017). Metatranscriptomic contigs were annotated in order to separate cnidarian and symbiont transcripts.

Splitting of the Transcripts and Transcriptome Completeness Estimation

Sequences from *M. complanata* and Symbiodiniaceae were classified by comparing two custom databases. A cnidarian database was constructed using the transcriptomes from

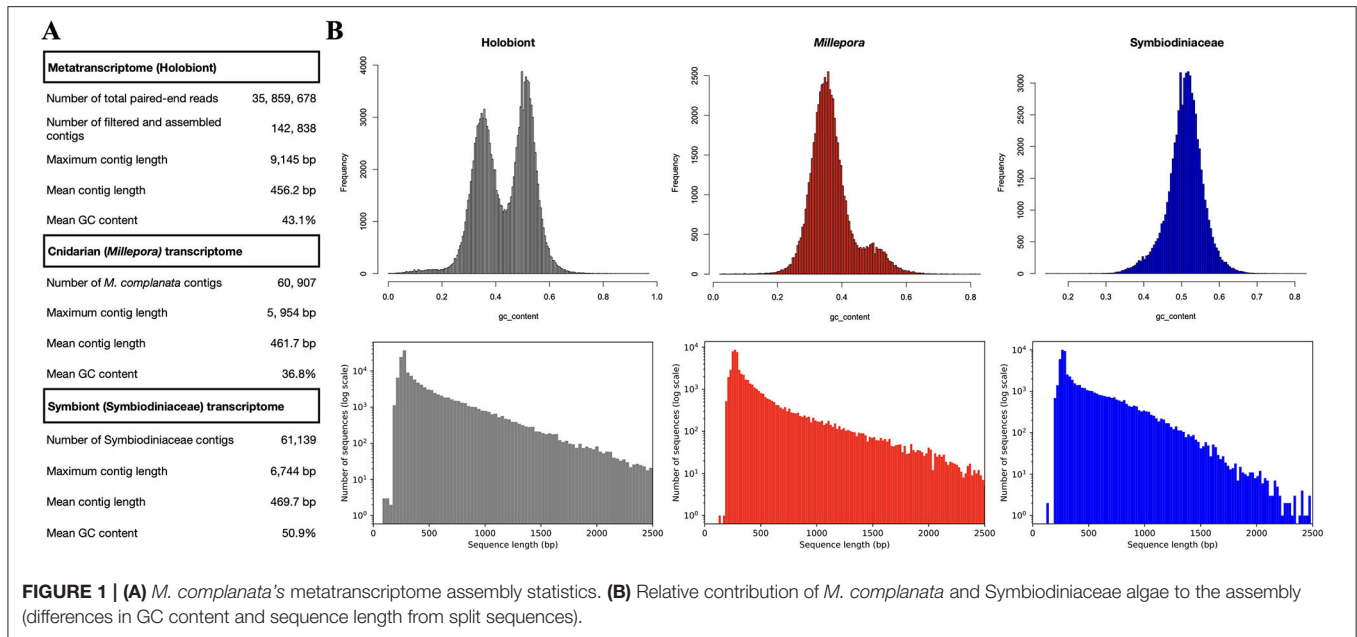


FIGURE 1 | (A) *M. complanata*'s metatranscriptome assembly statistics. **(B)** Relative contribution of *M. complanata* and Symbiodiniaceae algae to the assembly (differences in GC content and sequence length from split sequences).

Millepora alcornis (ID GFAS01, BIOPROJECT PRJNA344421), *Hydra vulgaris* (ID GGKF01, BIOPROJECT PRJNA399692), *Millepora squarrosa* (ID GFGU01, BIOPROJECT PRJNA350711), and *Nematostella vectensis* (ID HADP0, BIOPROJECT PRJEB13676). On the other hand, a database containing the following Symbiodiniaceae transcriptomes was created: *Symbiodinium* sp. (clade A1, ID GAKY01, BIOPROJECT PRJNA206215), *Symbiodinium* sp. (clade A2, ID GBGW01, BIOPROJECT PRJNA237994), *Breviolum* sp. (clade B2, ID GBRZ01, BIOPROJECT PRJNA260330), *Cladocopium* sp. (clade C, ID GBSC01, PRJNA260340), *Cladocopium* sp. (clade C, ID GAFO01, PRJNA175601), uncultured *Cladocopium* sp. (clade C, ID IADM01, BIOPROJECT PRJDB4715), *Durisdinium* sp. (clade D, ID GAFF01, BIOPROJECT PRJNA175600), *Durisdinium* sp. (clade D, ID GBRR01, BIOPROJECT PRJNA260333), uncultured *Durisdinium* sp. (clade D, ID IADN01, BIOPROJECT PRJDB4715), and *Symbiodinium muscatinei* (ID GFDR03, BIOPROJECT PRJNA359147). *M. complanata* metatranscriptome was compared against the customized cnidarian and Symbiodiniaceae databases using Blastn. Python scripts were used for splitting and retrieving confidently identified sequences (e-value threshold of $1.0E-20$) from *M. complanata* and Symbiodiniaceae. This procedure enabled assignment of 60,907 (42.6%) sequences to *M. complanata*, and 61,139 (42.8%) sequences to Symbiodiniaceae algae. Completeness of the separated transcriptome sequences was assessed with BUSCO using the Eukaryota database (Simão et al., 2015). Sequences assigned to Cnidaria possessed 75.3% of orthologs from Eukaryota core, whereas Symbiodiniaceae sequences displayed 51.8%. **Figure 1** shows the relative contribution of *M. complanata* and Symbiodiniaceae algae to the assembly. Statistics of split sequences showed that *M. complanata* sequences possessed 36.8% GC content and a maximum contig length of 5,954 bp, whereas symbionts' transcripts exhibited 50.9% GC content and a maximum contig

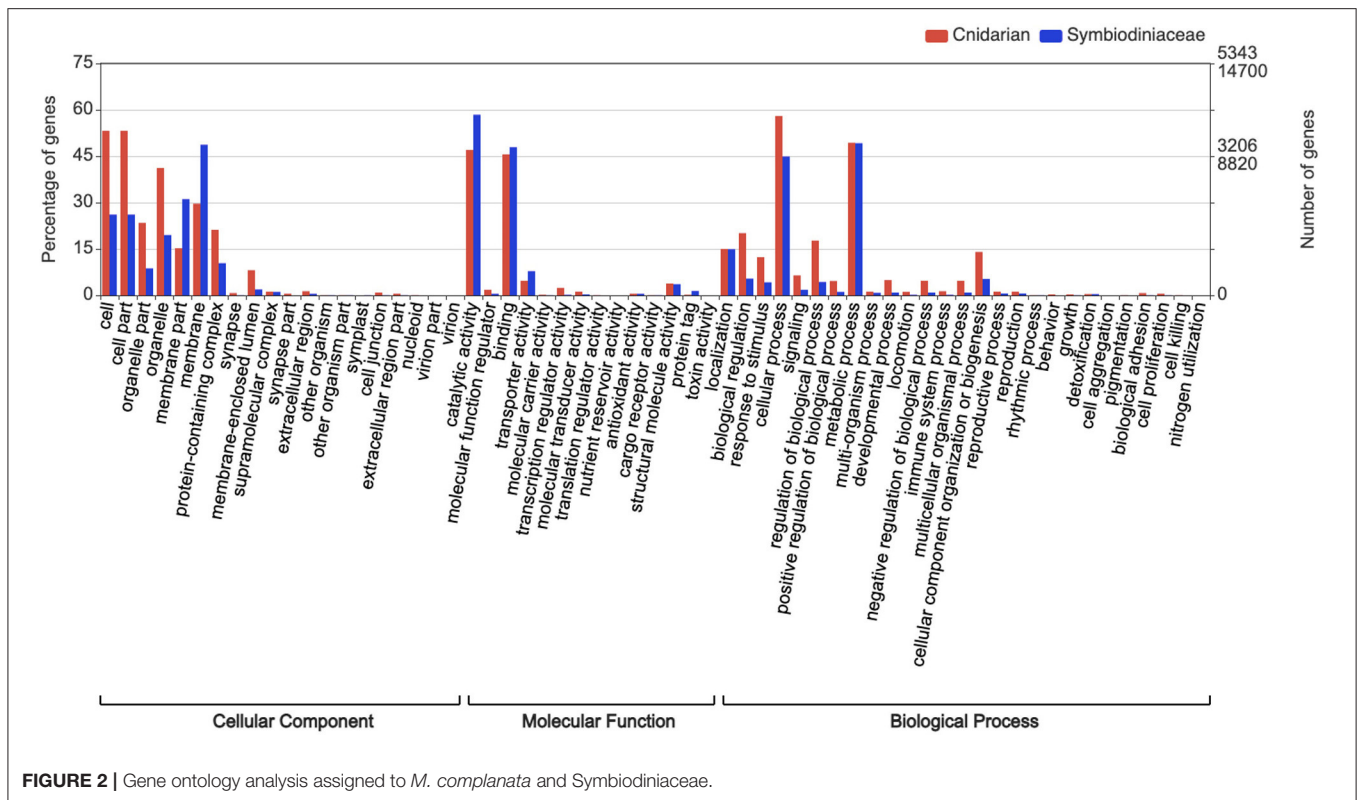
length of 6,744 bp (**Figure 1A**). GC content distribution and the length of split sequences are shown in **Figure 1B**. The assembled transcriptome was submitted to the Transcriptome Shotgun Assembly (TSA) project of NCBI. This TSA has been deposited in DDBJ/EMBL/GenBank under the accession GIXC00000000. The version described in this paper (GIXC01000000) is the first one.

Functional Annotation and Gene Ontology

All bioinformatic analyses were performed in the Databiology Lab platform (<https://www.lab.databiology.net/>). Sequences were annotated by sequence homology against the NCBI non-redundant database using Diamond (Buchfink et al., 2015) (<https://www.ncbi.nlm.nih.gov/>) (e-value threshold of $1.0E-6$). The annotation of transcripts for *M. complanata* and Symbiodiniaceae subsets are found in **Supplementary Material** (M_complanata_annotations.xlsx and Symbiont_annotations.xlsx). Gene Ontology (GO) terms were retrieved from Blastx hits with the Blast2GO software v. 5.2. Sequences were analyzed with "Mapping and Annotation." GO were grouped into Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) domains. GO annotations across sub-ontologies were visualized with WEGO 2.0 (<https://wego.genomics.cn>). The most over-represented GO terms across sub-ontologies for *M. complanata* were "cell" with 53.2% (CC), "catalytic activity" with 47% (MF), and "cellular process" with 58% (BP). Whereas, the most prevalent GO terms for Symbiodiniaceae were "membrane" with 48.7% (CC), "catalytic activity" with 58.4% (MF), and "metabolic process" with 49.2% (BP) (**Figure 2**).

Taxonomic Analysis

The assembled metatranscriptome was compared against the NCBI non-redundant database (<https://www.ncbi.nlm.nih.gov/>)



using Diamond (Buchfink et al., 2015) (e-value threshold of 1.0E-6) and MEGAN v. 6.19.8 was employed to perform the taxonomic analysis (Huson et al., 2016). Taxonomy assignments resulted in 61,604 hits, most of them were assigned to Dinophyceae (41.2%), Cnidaria (16.8%), and Proteobacteria (3.8%), the remaining percentage (37.2%) corresponded to non-dominant phyla such as Bacteroidetes, Actinobacteria, Firmicutes, Haptista, Ascomycota, Microsporidia, Chordata, Nematoda, Arthropoda, Mollusca, Placozoa, Ciliophora, Perkinsozoa, Bacillariophyta, Oomycota, Chlorophyta, and Streptophyta.

NOVELTY OF PRESENTED DATA

We describe, for the first time, the metatranscriptome of the *M. complanata* holobiont. After assignment of transcripts, cnidarian and symbiont transcriptomes were classified either as *Millepora* (42.6%) or Symbiodiniaceae (42.8%). Over-represented Dinophyceae hits imply a dominance of symbiotic algae sequences, which indicates that Symbiodiniaceae algae represent a common characteristic of hydrocorals' holobionts. The information obtained from *M. complanata* metatranscriptome will be a key piece for understanding the cellular survival processes, phylogeny, and physiology of milleporids from the class Hydrozoa.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories

and accession number(s) can be found at: <https://www.ncbi.nlm.nih.gov/>, PRJNA524427 and <https://www.ncbi.nlm.nih.gov/genbank/>, GIXC00000000.

AUTHOR CONTRIBUTIONS

VH-E: conceptualization, investigation, formal analysis, and writing-original draft preparation. NO-L and RH-M: investigation. JC-P: supervision, formal analysis, review, and editing. CI-A: supervision, review, and editing. AR-M: conceptualization, project administration, funding acquisition, review, and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.566913/full#supplementary-material>

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Conflict of Interest: JC-P was employed by the company Databiology Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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