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NOTE

Novel reference transcriptomes for the sponges *Carteriospongia foliascens* and *Cliona orientalis* and associated algal symbiont *Gerakladium endoclionum*

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Abstract Sponge transcriptomes are important resources for studying the stress responses of these ecologically important filter feeders, the interactions between sponges and their symbionts, and the evolutionary history of metazoans. Here, we generated reference transcriptomes for two common and cosmopolitan Indo-Pacific sponge species: *Carteriospongia foliascens* and *Cliona orientalis.* We also created a reference transcriptome for the primary symbiont of *C. orientalis—Gerakladium endoclionum.* Assemblies for *C. foliascens*, *C. orientalis*, and *G. endoclionum* contained 67,304, 82,895, and 28,670 contigs, respectively. Contigs represented 15,248–37,344 isogroups (~ genes) per assembly, and N50s ranged from 1672–4355 bp. Sponge transcriptomes were high in completeness and quality, with an average of 93% of core EuKaryotic Orthologous Groups (KOGs) and 98% of single-copy metazoan core gene orthologs identified. The *G. endoclionum* assembly was partial with 56% of core KOGs and 32% of single-copy eukaryotic core gene orthologs identified. These reference transcriptomes provide a

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valuable resource for future research assessing sponge stress responses.

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Introduction

Sponges, phylum Porifera, are one of the oldest lineages of multicellular animals (Feuda et al. 2017); hence, investigating the transcriptomes of different sponge species can provide insight into the evolution of metazoans and their gene expression profiles. Sponges have an uncertain future in the face of global climate change (see Bell et al. 2013; IPCC 2014) as well as local stressors including coastal development and increased runoff of nutrients, pesticides and sediments (Kroon et al. 2012; Stender et al. 2014). Transcriptomic analysis of sponges that have been exposed to different environmental conditions would improve our understanding of molecular stress response pathways and enhance our ability to effectively manage these ecologically important filter feeders (e.g., Koutsouveli et al. 2020). Although there are approximately 9,000 described sponge species (Van Soest et al. 2020), relatively few species have published genomes or transcriptomes (e.g., Riesgo et al. 2014a, b).

Here, we assembled the transcriptomes of two common and widely distributed Indo-Pacific sponge species—*Carteriospongia foliascens* and *Cliona orientalis*. Both are emerging model species that have been used extensively to study the physiological and ecological effects of environmental stressors on sponges (e.g., Pineda et al. 2016, 2017a, b, c). While both *C. foliascens* and *C. orientalis* host diverse populations of bacterial symbionts (see Pineda et al. 2016), *C. orientalis* additionally hosts an abundant population of eukaryotic Symbiodiniaceae: *Gerakladium endoclionum* (LaJeunesse et al. 2018), which comprises up to 96% of its algal symbiont community (Ramsby et al. 2018). We used sequences generated from the *C. orientalis* holobiont, i.e., host and symbiont, to construct a partial reference transcriptome for *Gerakladium endoclionum*. Matching host and symbiont transcriptomes provide a valuable tool to understand the holobiont response to changing environmental conditions and determine the cause–effect pathways for declining host health with environmental change.

Materials and methods

Samples and sequencing

Samples of *C. foliascens* and *C. orientalis* were collected in May 2015 from Fantome Is. (S 18°41.028′ E 146° 30.706) and Pelorus Is. (S 18° 32.903′ E 146° 29.172′), respectively, in the Great Barrier Reef under permits G12/35,236.1 and G13/35,758.1. Since *C. orientalis* is a bioeroding sponge, ten *C. orientalis* drill cores ($\sim$ 5 cm in diameter) were collected from a single individual growing on a dead colony of *Porites* sp. An individual of *C. foliascens* was cut into ten pieces (see Pineda et al. 2016). Sponges were healed and acclimated under natural light and flow-through seawater for 4 weeks before experiments were performed.

To capture the full range of gene expression responses, sponges were subjected to five different treatments at the Australian Institute of Marine Science (AIMS) National Sea Simulator: (i) decreased salinity, (ii) elevated temperature, (iii) elevated suspended sediment concentrations (SSCs) and sediment deposition, (iv) light attenuation, and (v) no stress control (see Supplemental for details). One genotype was used per species across all treatments to control for genetic variation. Two clones of each species were used for each treatment. Immediately after each treatment, $\sim$ 1 cm^3 of tissue from each clone was frozen in liquid nitrogen and stored at − 80°C (Riesgo et al. 2012).

Approximately 50 mg of each clone was ground using a mortar and pestle under a thin layer of liquid nitrogen to limit RNA degradation. All tools were rinsed in ethanol followed by RNase Zap (Sigma-Aldrich, USA) to remove contamination and deactivate RNA degrading enzymes. Total RNA was isolated using the Zymo ZR RNA miniprep kit, with in-column DNase digestion, and cleaned using the Zymo RNA Clean and Concentrator kit (Zymo Research, USA). Total RNA quality was checked using gel electrophoresis and quantified using a Quant-iT RiboGreen Assay (Thermo Fisher Scientific, USA). For each sponge species, RNA from individual treatments was combined in equal amounts from all sponge clones to a final total RNA concentration of 93 ng μl^{-1} for *C. foliascens* and 160 ng μl^{-1} for *C. orientalis*. To isolate eukaryotic messenger RNA (mRNA), a TruSeq Stranded mRNA-seq sample prep was performed. The mRNA was then sequenced across two Illumina HiSeq2500 lanes at the Ramaciotti Centre for Genomics (University of New South Wales, Australia), generating 2 × 100 base pair (bp) paired-end (PE) rapid runs.

Transcriptome assembly and annotation

Reads were trimmed using publicly available scripts (Matz 2015; Meyer 2016) and assembled with Trinity v 2.8.5 (Grabherr et al. 2011) following established protocols (Kenkel and Bay 2017, see Supplemental Material and the github repository below for a full description). After assembly, additional quality control was performed to ensure that only target transcripts, i.e., derived from *C. foliascens*, *C. orientalis* or *G. endoclionum*, were included in their respective reference transcriptomes using protocols outlined by Kitchen et al. (2015) and Kenkel and Bay (2017). These quality controls also removed short contigs, ribosomal and mitochondrial RNA contamination (see Supplemental Material). Within each of the three transcriptomes, contigs were assigned to isogroups (∼ genes) and assigned gene names, gene ontologies (GO) (Gene Ontology Consortium 2000) and Kyoto Encyclopedia of Genes and Genomes (KEGG) IDs, following established protocols (Dixon 2015; Matz 2015b; Kenkel and Bay 2017; see Supplemental). The guanine-cytosine (GC) content of the transcriptomes was calculated using the BBMap package (Bushnell 2014). Transcriptome completeness was assessed by Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis (Simão et al. 2015) using metazoan or eukaryotic references for sponge and algal transcriptomes, respectively.

Results and discussion

In the decreased salinity and darkness treatments, *C. orientalis* visibly bleached after two days, but *C. foliascens* did not exhibit any color changes. Sponges were not visibly affected by sediment exposure or elevated seawater temperature. For *C. foliascens* and *C. orientalis*, respectively, RNA had average A260/A280 ratios of 1.88 and 2.02 and A260/A230 ratios of 1.11 and 1.67. The low quality of the latter RNA was likely due to proteinaceous contamination that appeared to be universal in this species across treatments but did not interfere with sequencing depth or quality. Sequencing produced 409 and 418 million raw reads for *C. foliascens* and *C. orientalis*, respectively (Table 1).The holobiont assemblies of *C. foliascens* and *C. orientalis* contained 225,126 (N50 = 1,284) and 146,510 (N50 = 1,949) contigs > 400 bp (Table 1). After partitioning, 67,304 and 82,895 contigs, for *C. foliascens* and *C. orientalis*, respectively, were considered the 'sponge-specific' transcriptome assemblies. The partitioned *G. endoclionum* transcriptome isolated from the *C. orientalis* holobiont comprised 28,670 contigs (Table 1). The *C. foliascens*, *C. orientalis*, and *G. endoclionum* transcriptomes contained 15,248, 37,344, and 21,566 isogroups, respectively, with mean lengths of 3,024, 1,756, and 1,375 bp (Table 1). The number of isogroups identified in the *C. foliascens* and *C. orientalis* transcriptomes was comparable to previously published sponge transcriptomes with ∼ 11,000–60,000 expressed genes (López-Maury et al. 2008; Riesgo et al. 2014a; Guzman and Conaco 2016). The *G. endoclionum* transcriptome was comparable in size to the *S. kawagutii* genome (36,850 genes, Lin et al. 2015) and other Symbiodiniaceae transcriptomes (23,777–26,986 genes, Ladner et al. 2012). The respective GC content of each assembly was 40.2, 45.5, and 59%, matching reported values for metazoans (35–55%, Riesgo et al. 2014b; Francis et al. 2017; Karimi et al. 2017) and Symbiodiniaceae (45–65%, Karimi et al. 2017). For *C. foliascens* and *C. orientalis*, the percentage of genes assigned a name or GO term was 64 and 77%, respectively (Table 1), comparable with other sponge transcriptomes (30–70%, Riesgo et al. 2014a). Only 39% of *G. endoclionum* isogroups could be assigned functions or GO term annotations; however, this is consistent with functional annotation of other intracellular Symbiodiniaceae transcriptomes (34–44%, Ladner et al. 2012). The isogroups for *C. foliascens*, *C. orientalis*, and *G. endoclionum* were assigned 3641, 5339, and 2191 unique KEGG annotations, respectively.

The transcriptomes for *C. foliascens* and *C. orientalis* were largely complete based on BUSCO analysis (92.8% and 94.2% complete, respectively) and the representation of nearly all core eukaryotic Orthologous Groups (KOGs) 97.9% and 98.7%, respectively (Table 1). BUSCO analysis of the transcriptome of *G. endoclionum* was 32.3% complete, and 56% of core KOGs were identified (Table 1). A reduced completeness (33–42%) in genomes isolated from intracellular Symbiodiniaceae also occurs in corals (Liu et al. 2018), perhaps due to poor representation of related phyla within the BUSCO eukaryotic gene set. The *G. endoclionum* transcriptome contained 86% more isogroups than the Symbiodiniaceae transcripts identified within the transcriptome assembly of the closely related sponge holobiont, *Cliona varians* (Riesgo et al. 2014b). Therefore, the current transcriptome for *G. endoclionum* was considered useful for future studies *in hospite*.

C. foliascens and *C. orientalis* are widely distributed throughout the Indo-Pacific and are ecologically important components of the reef ecosystem, particularly in the GBR where *C. foliascens* is a dominant component of the benthos and *C. orientalis* bioerodes calcium carbonate substrate, including the skeletons of live coral. These data represent a substantial contribution to publicly available poriferan genetic resources and will provide the framework needed to develop these two sponge species into models for field and laboratory studies, particularly research

Table 1 Assembly statistics for the de novo transcriptomes

	Carteriospongia foliascens holobiont	*Cliona orientalis* holobiont	
N raw reads ($\times 10^6$)	409	418	
N qual filtered: PE, SE ($\times 10^6$)	32.5, 2.9	50.5, 8.7	
N contigs holobiont	146,510	225,126	
	Carteriospongia foliascens	*Cliona orientalis*	*Gerakladium endoclionum*
N contigs target species only	67,304	82,895	28,670
Mean GC content target species only	40.2	45.5	59
N genes	15,248	37,344	21,566
Mean contig length (bp)	3024	1756	1375
N50 (bp)	4355	2369	1672
% Annotated	64	77	39
% Core KOGs	97.9	98.7	56
BUSCOs			
N complete (%)	908 (92.8)	921 (94.2)	98 (32.3)
N partial (%)	14 (1.48)	16 (1.94)	16 (5.28)
N missing (%)	56 (5.73)	38 (3.89)	189 (62.8)

examining the molecular mechanisms underpinning how reef sponges respond to environmental perturbation.

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Availability of supporting data All data can be accessed here: https://www.dropbox.com/sh/82ue5l16n4xzxww/AABENUi-Cdbm_z-6x4Gj3qICa?dl=0. Raw data have also been deposited on NCBI's SRA under accession numbers PRJNA639714 and PRJNA639798 for the *C. orientalis* holobiont and *C. foliascens*, respectively. The final, annotated assemblies as well as tabular files containing Kegg and GO information are also included in Supplemental Material. Scripts used throughout the assembly process can be found here: https://github.com/bstrehlow/Transcriptome_assembly/tree/master

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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