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Transcriptome sequencing and characterization of *Symbiodinium muscatinei* and *Elliptochloris marina*, symbionts found within the aggregating sea anemone *Anthopleura elegantissima*

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ABSTRACT

There is a growing body of literature using transcriptomic data to study how tropical cnidarians and their photosynthetic endosymbionts respond to environmental stressors and participate in metabolic exchange. Despite these efforts, our understanding of how essential genes function to facilitate symbiosis establishment and maintenance remains limited. The inclusion of taxonomically and ecologically diverse endosymbionts will enhance our understanding of these interactions. Here we characterize the transcriptomes of two very different symbionts found within the temperate sea anemone *Anthopleura elegantissima*: the chlorophyte *Elliptochloris marina* and the dinoflagellate *Symbiodinium muscatinei*. We use a multi-level approach to assess the diversity of genes found across *S. muscatinei* and *E. marina* transcriptomes, and compare their overall protein domains with other dinoflagellates and chlorophytes. Our analysis identified several genes that are potentially involved in mitigating stress response (e.g., heat shock proteins pathways for mediating reactive oxygen species) and metabolic exchange (e.g., ion transporters). Finally, we show that *S. muscatinei* and other *Symbiodinium* strains are equipped with a high salt peridinin-chl-protein (HSPCP) gene previously identified only in free-living dinoflagellates. The addition of these transcriptomes to the cnidarian-symbiont molecular toolkit will aid in understanding how these vitally important symbiotic relationships are established and maintained across a variety of environmental conditions.

1. Introduction

The aggregating sea anemone *Anthopleura elegantissima*, a dominant, long-lived intertidal species found along the western coast of North America, is capable of hosting two distinct endosymbionts: a chlorophyte (zoochlorellae, *Elliptochloris marina*) and a dinoflagellate (zooxanthellae, *Symbiodinium muscatinei*). These two symbionts exhibit strikingly different growth rates and physiological profiles that likely affect their biotrophic contribution to the sea anemone host (Bergschneider and Muller-Parker 2008; Dimond et al., 2013). *E. marina* can be found at up to 4 times greater density in *A. elegantissima* and grow up to 8 times faster than *S. muscatinei*; however, they have about half the volume, half the carbon content, and less chlorophyll per cell (Bergschneider and Muller-Parker 2008). In terms of carbon translocation, *S. muscatinei* is the more productive symbiont, being up to 2.5 times more productive than *E. marina* during the summer months (Bergschneider and Muller-Parker 2008) and able to translocate up to 5 times more carbon to the host (Verde and McCloskey 1996a).

Symbiont species that are capable of translocating substantial portions of their photosynthate to the host have been hypothesized to augment their hosts' nutrient intake to increase reproductive output (Davy et al., 2012). Anemones hosting *E. marina* tend to reproduce sexually, while individuals with the more productive *S. muscatinei* tend to clone by fission (Bingham et al., 2014). By supporting a clonal reproductive strategy in its host, *S. muscatinei* likely makes a significant contribution to the abundance and persistence of *A. elegantissima*, and itself, in upper intertidal habitats where it may be a spatially dominant species. Alternative reproductive strategies may be influenced by the products the symbionts provide the host: *E. marina* translocates mainly amino acids (Minnick 1984) and *S. muscatinei* translocates glycerol and sugars (Trench 1971). *A. elegantissima* hosting *S. muscatinei* exhibit greater concentrations of most fatty acids than do *A. elegantissima* hosting *E. marina* (Quesada et al., 2016).

The symbiont assemblage in *A. elegantissima* is largely determined

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by latitude and position along a tidal gradient. At higher latitudes (e.g., Oregon to Alaska), stable symbioses are established with either *S. muscatinei* or *E. marina*, while at lower latitudes (e.g., Oregon to Baja California) *S. muscatinei* is the primary symbiont (Secord and Augustine 2000). Where both symbionts can be found associated with *A. elegantissima*, their vertical distribution along intertidal gradients is superimposed on these biogeographic clines (Secord and Augustine 2000). *A. elegantissima* subjected to prolonged high-temperature (> ~ 20°C) and irradiance (> ~ 1000 μmol quanta $m^{-2} s^{-1}$) characteristic of shallower intertidal areas are primarily dominated by *S. muscatinei* while individuals hosting *E. marina* are restricted to cooler, shaded sites, generally lower in the intertidal zone (Bates et al., 2010; Dimond et al., 2011; Secord and Augustine 2000; Secord and Muller-Parker 2005).

Significant gains have been made in understanding eukaryotic symbioses by integrating physiological mechanisms with molecular signaling (Davy et al., 2012). The symbiont-host interactions between *A. elegantissima* and its symbionts (*E. marina* or *S. muscatinei*) have provided insight into physiology and nutrient exchange of this symbiosis, but our understanding of genomic mechanisms involved with metabolic contributions from the symbionts has been limited due to a lack of complete transcriptomes needed to complement what is currently available for the anemone host (Kitchen et al., 2015; Macrander et al., 2015; Richier et al., 2008; Rodriguez-Lanetty et al., 2006). Representative transcriptomes for both hosts and symbionts are necessary to conduct metatranscriptomic analyses to better characterize how these biological relationships form and are maintained.

Here we use an RNA-Seq approach to assemble de novo transcriptomes of both *E. marina* and *S. muscatinei* isolated from *A. elegantissima*. The newly sequenced transcriptomes complement existing genomic resources for predominantly tropical symbiotic and free-living dinoflagellates (e.g., Aranda et al., 2016; Bayer et al., 2012; Lin et al., 2015; Parkinson et al., 2016), in addition to providing a transcriptome for the chlorophyte *E. marina*. Symbiotic unicellular chlorophytes are poorly represented in genomic databases, yet they are common to well-studied symbioses in *Hydra* (*Chlorella*) and lichens (Kovacevic 2012). Here we report the overall transcriptomic diversity in each symbiont and contrast that with other endosymbiont studies, in addition to exploring the genetic diversity behind potential molecular mechanisms involved with stress response and metabolic exchange. The characterization of these two symbiont transcriptomes will help elucidate processes that shape life history traits in sea anemone hosts and provide significant insight into the molecular signals involved in this dynamic symbiotic relationship.

2. Materials and methods

2.1. Symbiont isolation and sequencing

Primarily zooxanthellate (*S. muscatinei*-bearing) and primarily zoochlorellate (*E. marina*-bearing) polyps of *A. elegantissima* were collected from Point Lawrence, Orcas Island, Washington, USA, and placed in a seawater table at the Shannon Point Marine Center (SPMC) in Anacortes, Washington (Table 1). On 9 July 2016, ~ 12 tentacles from three anemones for each symbiont type were removed and homogenized in 5 μm filtered seawater (FSW) in a small blender and 15 ml of the fresh homogenate was centrifuged (1500*g* for 3 min) to pellet the algal symbionts. The supernatant was discarded and the remaining algal pellet was washed three times by repeated resuspension and centrifugation in FSW (1500*g* for 3 min), then filtered through 100 μm Nitex mesh to remove any clumps. RNALater (Ambion) was added to the isolated symbionts and shipped to the University of North Carolina, Charlotte for RNA extraction and sequencing. A second set of samples were isolated by NaOH treatment (Zamoum and Furla 2012), but the lower quality and quantity of RNA did not permit further processing of these samples.

Table 1
Transcriptomic and environmental features

Item name	Definition
Transcriptome analysis investigation type	Eukaryote
Species	*Symbiodinium muscatinei* and *Elliptochloris marina*
Project name	*Anthopleura elegantissima* Symbiont Transcriptomes
Geographic location (latitude and longitude)	48°39′38″ N, 122°44′32″ W
Geographic location (country and/or sea, region)	Rosario Strait, Salish Sea
Collection date	July 6, 2015
Environment (biome)	Marine - Upper Intertidal
Environment (feature)	Rocky Shore
Environment (material)	Symbiont found within laboratory animals maintained in aquarium provided with flowing ambient seawater
Observed biotic relationship	Symbiont
Trophic level	Autotroph
Sample collection method	Mechanically separated from host tentacles.
Temperature	11 °C
Salinity	30 ppt
Sequencing	
Nucleic acid extraction	PureLink RNA Mini Kit (Ambion)
Nucleic acid amplificaiton	HiSeq PE cluster Kit v4
Library size (reads)	(1) *S. muscatinei:*105,018,781 (2) *E. marina:* 90,663,858
Library construction method	Paired-end (125 bp PE)
Sequencing method	Illumina HiSeq 2500
Sequencing quality check	FastQC
Transcriptome assembly data	
Assembly method	De novo using Trinity
Assembly name	(1) *S. muscatinei* transcriptome (2) *E. marina* transcriptome
Finishing strategy	Draft
Annotation source	Blast2GO; REVIGO

For the mechanically-isolated symbionts, the RNALater (QIAGEN) was replaced with lysis buffer from PureLink RNA Mini Kit (Ambion), with the symbiont cells macerated in the MiniBeadBeater-16 (BioSpec Products, Model 607) at 3450 RPM using 0.5 mm glass beads (BioSpec Products). Total RNA was extracted using the PureLink RNA Mini Kit (Ambion) following the manufacturer's protocol, including the optional DNAse treatment step to minimize DNA contamination. The quantity and quality of the RNA extraction was quantified on the Nanodrop ND-2000 (Thermo Scientific) and the Bioanalyzer 2100 (Agilent). The RNA-Seq libraries were prepared at the David H. Murdock Research Institute (DHMRI) (Kannapolis, NC) using the HiSeq PE cluster Kit v4, with paired end (2 × 125 bp libraries) sequencing on the Illumina Hiseq 2500. Overall quality of the raw reads was evaluated using the program FastQC (Andrews 2010).

2.2. Transcriptome assembly and bioinformatic analyses

Prior to transcriptome assembly, raw reads were cleaned using the program Trimmomatic (Bolger et al., 2014), removing Illumina barcodes, leading and trailing five bases, reads below 25 bases, and the trailing portions of reads with a sliding window of 4 bases having a quality score lower than 5. Both transcriptomes were assembled in the program Trinity (Grabherr et al., 2011) with default parameters. Relative expression levels for each transcriptome were calculated using the program RSEM (Li and Dewey 2011) within the Trinity programming suite (Grabherr et al., 2011). As low levels of contamination and incomplete transcripts may result in an overabundance of unwanted or

mis-identified transcripts in downstream analyses, transcripts expressed at low levels (FKPM < 1.0) and fewer than 200 bases were removed from each symbiont transcriptome. Additionally, transcripts sharing high sequence similarity (> 90%) were clustered using CD-HIT-EST (Li and Godzik 2006), as there are typically higher numbers of predicted transcripts in these types of de novo assemblies (Audoux et al., 2017).

To determine if background populations of each symbiont type or their cnidarian host were a potential source of contamination in each dataset, nucleotide BLAST searches were performed against predicted protein databases derived from the sea anemone *Nematostella vectensis* genome (Putnam et al., 2007), the dinoflagellate *S. minutum* genome (Shoguchi et al., 2013), and the *Coccomyxa subellipsoidea* genome (Blanc et al., 2012). To allow for the *E. marina* screening to be more inclusive, proteins from Chlorophyta were downloaded from UniProt (accessed 6–23-2017), as the phylogenetic position of *E. marina* as it relates to other chlorophytes has not been thoroughly evaluated. For each transcriptome, transcripts were retained only if that transcript had the greatest e-value for its respective symbiont lineage. The transcriptomes were then screened against predicted proteins from the *Sacchromyces cerevisiae* genome (Cherry et al., 2012) in order to screen potentially contaminated transcripts with a fungal origin. The remaining transcripts were considered symbiont-specific assemblies, although there was still some potential for contamination from other taxa.

Each symbiont transcriptome was used in a Blast2GO analysis (Conesa et al., 2005) to determine which gene ontology (GO) functional groups were found in the highest abundance for each transcriptome. The overall representation for different gene ontology groups was summarized using REVIGO (Supek et al., 2011) with allowed similarity at 0.5 and SimRel as the semantic similarity measure. In addition to the BLAST2GO analysis for the most abundant transcripts, the transcriptomes were translated into their predicted open reading frame using Transdecoder (Haas et al., 2013), with the protein domains screened using HMMER (version 3.1b2) (Eddy 1996) against the Pfam database (version 31.0). Our Pfam domain counts were then compared to the diversity previously reported in other dinoflagellates (Aranda et al., 2016). Protein sequences from five chlorophyte JGI genome projects: *Ostreococcus lucimarinus* (Palenik et al., 2007), *Ostreococcus* sp. RCC809, Coccomyxa_C169 (Blanc et al., 2010), *Symbiochloris reticulata*, and *Chlamydomonas reinhardtii* (Merchant et al., 2007) were also clustered at 90% identity using CD-HIT-EST (Li and Godzik 2006) and included in our Pfam analysis.

Genes of interest from previously published *Symbiodinium* or dinoflagellate-focused studies were used to identify transcripts potentially involved in thermal tolerance (Gierz et al., 2017; Levin et al., 2016) and the exchange of metabolic products (Aranda et al., 2016; Butterfield et al., 2016; Gierz et al., 2017). Candidate transcripts were identified using BLAST searches of candidate functional genes (Supplemental File 1) against the *S. muscatinei* and *E. marina* transcriptomes. As there was potential contamination from the anemone host or other symbionts in our final transcriptome assemblies, we confirmed genes of interest with their designated organism via reciprocal BLAST hits against NCBI's non-redundant protein database. For certain genes of interest, alignments were done using MAFFT v7.017 (Katoh et al., 2002) in Geneious v 7.1.9 (Kearse et al., 2012). Gene tree reconstructions were done using the program FastTree2 (Price et al., 2010) with 1000 resampling bootstrap replicates in PHYLIP's Seqboot (Felsenstein 2005).

3. Results and discussion

3.1. Transcriptome assembly

The resulting Illumina paired-end run produced 105,018,781 paired-end reads for *S. muscatinei* and 90,663,858 paired-end reads for *E. marina*. Following the Trimmomatic cleanup, 92.72% of the paired-end reads for *S. muscatinei* were retained, with 7.11% of the forward-only reads retained, 0.02% of the reverse-only reads retained, and 0.14% of the reads dropped. For *E. marina*, 93.22% of the paired-end reads were retained, 6.68% of the forward-only reads were retained, 0.03% of the reverse-only reads were retained, and 0.08% of the reads were dropped (Table 2). From the retained reads, the Trinity assembly resulted in 322,112 transcripts and 234,789 Trinity "genes" for *S. muscatinei*. The assembly for *E. marina* was notably smaller, with 232,144 transcripts and 170,497 Trinity "genes" (Table 2). Once the transcriptomes were subjected to our bioinformatic pipeline, which included a minimum expression threshold (FKPM > 1.0), minimum sequence length (200 bp), similarity clustering (> 90% similarity), and lineage specific BLAST screening, the transcriptomes for *S. muscatinei* and *E. marina* were left with 50,576 and 10,966 transcripts, respectively (Table 2).

Table 2
Transcriptome assembly and assessment

	S. muscatinei	*E. marina*
Trimmomatic		
Both	97,375,118	84,512,425
Forward Only	7,469,050	6,054,285
Reverse Only	23,360	23,162
Dropped	151,253	73,986
Trinity Assembly		
Bases	290,506,423	202,998,599
Transcripts	322,112	232,144
'Genes'	234,789	170,497
N50	1574	1629
Assembly cleanup (FKPM > 1.0, Length > 200)		
Transcripts	99,314	58,971
CD-HIT-EST (90% similarity)		
Transcripts	75,300	45,272
BLAST screening and final transcriptome statistics		
Bases	65,518,596	10,837,850
Transcripts	50,576	10,966
Trinity 'genes'	44,815	10,352
N50	1705	1434
Max. Length	15,861	23,340
Average	1295	988
Percent GC	51.78%	50.38%
BLAST2GO (BLAST and InterProt)		
Sequences w/GO Hits	17,537	3220

Ultimately, the number of transcripts retained for the *S. muscatinei* fell within the typical range of previously reported Clade B *Symbiodinium* transcriptomes (e.g., Parkinson et al., 2016). As there are no transcriptome datasets available for Chlorophytes from the genus *Elliptochloris*, it is difficult to accurately predict the size of their transcriptome. *Coccomyxa* and *Elliptochloris* are both members of the class Trebouxiophyceae, however, *Coccomyxa* may not accurately represent the complexity present in the *E. marina* genome. For the more closely-related *Coccomyxa* C169 and the *Hydra* symbiont *Chlorella variabilis* NC64A, there are 9629 and 9791 predicted proteins, respectively (Blanc et al., 2010, 2012). These numbers are slightly lower than ours, however, the similarity in numbers and our pipeline removal of over 95% of the original transcripts has us confident that this final transcriptome is primarily *E. marina*.

3.2. Data accessibility

RNA Sequences, sample name: Sm_1
NCBI SRA: SRR5133333
BioProject: PRJNA359147
BioSample Acession number: SAMN06187570
TSA Accession number: GFDR00000000
RNA Sequences, sample name: Em_1
NCBI SRA: SRR5133334
BioProject: PRJNA359147

BioSample Acession number: SAMN06187571
TSA Accession number: GFDT00000000

3.3. Functional annotation

The Blast2GO analysis recovered BLAST hits associated with gene ontology terms for 17,537 (48%) of the *S. muscatinei* transcripts, when searching against the Cloud-Based Blast2GO database (Table 2). In total for each functional domain there were 8821 biological process, 4003 molecular function, and 1453 cellular component GO groups (Supplemental Table 1). For the *E. marina* transcriptome, the Cloud-Based Blast2GO database identified gene ontology groups in association with 3220 transcripts (29%) resulting in 3443 biological process, 1500 molecular function, and 787 cellular component GO groups for each functional domain (Supplemental Table 2).

For each transcriptome, the REVIGO analysis summarized the overall gene ontology abundance of the molecular function and biological process domains into 350 representative groups. For the biological process domain, both transcriptomes had oxidative-reduction process as the most abundant GO group. The *S. muscatinei* transcriptome also had a high representation of metabolic process, protein phosphorylation, multicellular organism development, cell cycle, and protein transport. The *E. marina* transcriptome had some of these (e.g., metabolic process and protein phosphorylation), but were proportionately less abundant in *E. marina* when compared to *S. muscatinei* (Fig. 1).

For the molecular function domain, both transcriptomes had protein binding as the most abundant gene ontology group, in addition to nucleotide binding/nucleic acid binding and RNA binding, although these were proportionately higher in the *S. muscatinei* transcriptome (Fig. 1). Within the molecular function domain, the transcriptomes differed with regard to enzymatic activity, with hydrolase activity and transferase activity in greater abundance for *S. muscatinei* and oxidoreductase activity and catalytic activity proportionately more abundant in the *E. marina* transcriptome (Fig. 1). It is worth noting that these GO groups represent < 50% of each transcriptome and there is a notable difference between abundance measures as significantly more GO groups were recovered for *S. muscatinei* than *E. marina*. Additionally, the taxonomic representation in the reference BLAST2GO database is not entirely taxonomically inclusive, which may be a limiting factor in our analysis.

The overall gene ontology analysis produced similar results across both transcriptomes when considering the most abundant gene ontology groups, however, there are some subtle differences between the less abundant groups that are worth noting. The oxidation-reduction process plays a significant role in cnidarian-coral symbiosis and, when disrupted through thermal or other stressors, may result in bleaching (Desalvo et al., 2008; Downs et al., 2013; Lesser 2006). Additionally, protein phosphorylation has also been shown to aid in redox sensing and regulation in chloroplast thylakoids (Allen et al., 1981; Dalle-Donne et al., 2001; Grieco et al., 2016; Vener et al., 1998). The larger number of transcripts from *S. muscatinei* associated with these processes may be indicative of their inherent ability to accommodate higher temperatures (> ~ 24°C) without succumbing to oxidative stress (Muller-Parker et al., 2007). It is worth noting, with the exception of multicellular organism development and cell cycle in the biological process domain and nucleotide binding and nucleic acid binding in the molecular function domain (Fig. 1), our recovered GO groups differed significantly from another *Symbiodinium* strain grown across variable irradiation conditions (Xiang et al., 2015). But these differences could have arisen through alternative assembly and annotation approaches as we recovered drastically more GO terms.

Within the biological process domain for *S. muscatinei*, the remaining abundant gene ontology groups may be associated with this symbiont's ability to produce and transfer metabolites and other energy

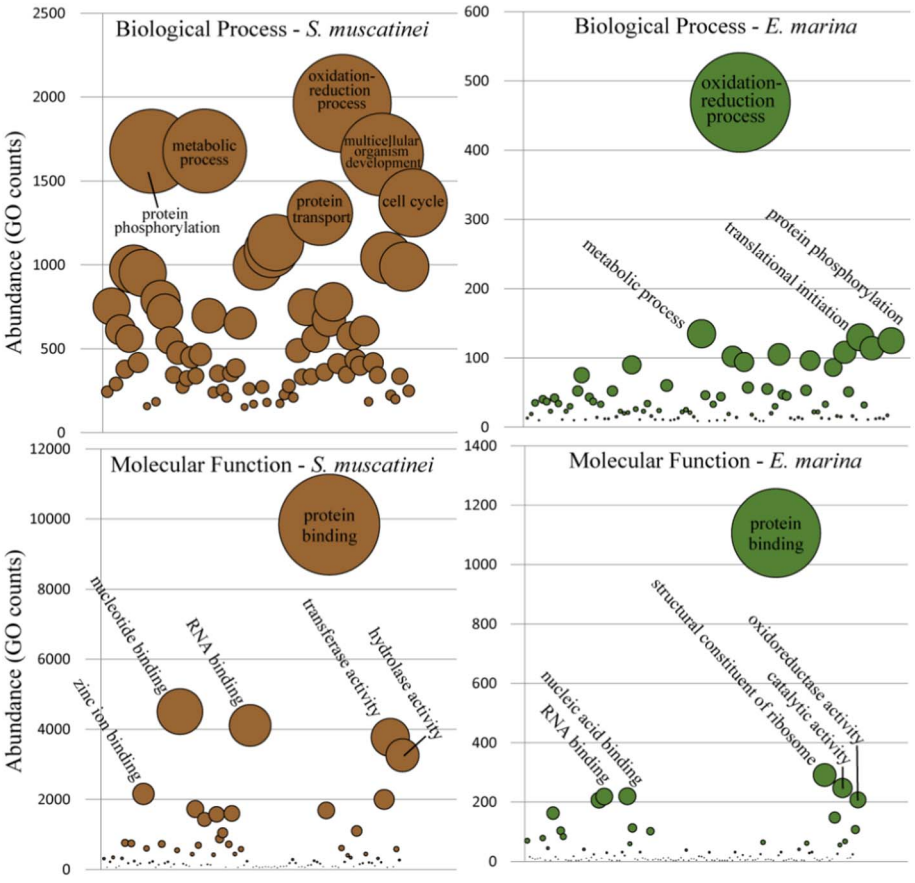

Fig. 1. Gene ontology analysis of most abundant groups for each transcriptome in the biological process and molecular function domains. The x-axis represents REVIGO gene ontology semantic similarities, where more similar GO groups are found adjacent to one another. The y-axis and bubble size are both GO group abundance calculations provided by REVIGO. The most abundant GO groups in each analysis are labeled.

sources to the host. Although multicellular organism development seems counterintuitive for unicellular organism, the REVIGO analysis grouped transmembrane transport [GO:0055085] and transcription, DNA-templated [GO:0006351], within this group. These gene ontology groups, in addition to metabolic process and protein transport, may be associated key proteins that have permitted *S. muscatinei* to be the more efficient and productive symbiont associated with *A. elegantissima* (Bergschneider and Muller-Parker 2008; Verde and McCloskey 1996a). The proportionately higher abundance of the cell cycle domain in association with *S. muscatinei* seems counterintuitive as *E. marina* exhibits a faster growth rate (Bergschneider and Muller-Parker 2008). This, along with the abundance of multicellular organism development in *S. muscatinei* (Fig. 1), may simply be a byproduct of the limitations to our overall GO approach. The molecular function domain appears to be more similar between the two symbionts, differing only in the representative enzymatic activity GO groups. The majority of the GO groups identified here could be associated with multiple proteins involved with antioxidant response, molecular chaperones, and fatty acid desaturases (Gierz et al., 2017). Although some of these resemble metabolic (transferase activity) or stress response categories (oxidoreductase activity), future experiments that can quantify the expression of the potentially functionally important genes will be necessary to determine what these transcripts may be doing.

Overall, our Pfam analysis found 10,252 different Pfam domains across both symbiont transcriptomes, with notable differences in Pfam abundances for *S. muscatinei* and *E. marina*. The most abundant Pfam domains in *S. muscatinei* corresponded to a variety of Ef-hand motifs (Supplemental Table 3), which is a motif found in a large family of calcium-binding proteins. Although a calcium-binding protein has been identified in *A. elegantissima* (Hauck et al., 2007), those proteins remain largely unexplored in dinoflagellates (Ganot et al., 2011). Several other protein-binding domains were found in high abundance in *S. muscatinei*. These included ankyrin repeats and tetratricopeptide repeats (Supplemental Table 3), which also remain largely uncharacterized in dinoflagellates (Hamada et al., 2013; Zhang et al., 2014). Even less is known about *Elliptochloris* and its relatives. There does not seem to be an overabundance of any specific Pfam domains within the *E. marina* transcriptome, however, the most abundant Pfam domain links to a pentatricopeptide repeat, a conserved domain structure involved with chloroplast RNA editing (Kotera et al., 2005; Schmitz-Linneweber et al., 2005). Collectively our bioinformatic analysis did not identify any distinct Pfam domains or functionally-relevant proteins that may provide insight into which proteins play a significant role in the survival, proliferation, and growth of *E. marina*, an atypical cnidarian symbiont.

To better understand how the abundance of functionally important proteins differs in *S. muscatinei* and *E. marina* relative to other *Symbiodinium* species, we compared previously reported Pfam domain counts (Aranda et al., 2016) with our transcriptomes (Supplemental Table 3). Among the 81 protein domains that exhibited significant differences across *Symbiodinium* species (see Aranda et al., 2016), we recovered 80 and 72 Pfam domains for *S. muscatinei* and *E. marina*, respectively. Hierarchical clustering of these protein domains grouped *S. muscatinei* with *S. microadriaticum* and *S. minutum*, while *E. marina* was placed within a cluster containing free living dinoflagellates (Fig. 2).

The overall Pfam domain count and hierarchical placement of *S. muscatinei* in our analysis were not surprising. These symbionts are all closely-related members of the same genus (*Symbiodinium*), and live in hosts that thrive under higher irradiance ($> \sim 1000$ μmol quanta $m^{-2} s^{-1}$). Their Pfam domain could be representative of their shared evolutionary history, so it is difficult to determine if their placement is due exclusively to their preferred high-irradiance habitat. To some degree, the separation of *S. kawagutii* from these other species of *Symbiodinium* was expected (see Fig. 2Aranda et al., 2016; Thornhill et al., 2017), but the placement most closely to *E. marina* was a surprise. The close association between *E. marina* and *S. kawagutii* could be attributed to symbiotic convergence (Fan et al., 2012), however, strong support for *S. kawagutii* as an intracellular symbionts is currently lacking (Thornhill et al., 2017; Yuyama et al., 2005). Although *E. marina* does proportionately share some high Pfam counts with other member of *Symbiodinium* (Fig. 2), Pfam counts for *S. muscatinei*, *S. microadriaticum*, and *S. minutum* were much higher overall across the majority of these Pfam domains (Supplemental Table 4).

Alternatively, the convergence observed between *E. marina* and these functionally important Pfam domains in *S. kawagutii* may be reflective of a preference of *E. marina* for lower irradiance ($< \sim 1000$ μmol quanta $m^{-2} s^{-1}$) environments (Dimond et al., 2011; Iglesias-Prieto and Trench 1994, 1997; Secord and Muller-Parker 2005). Xiang et al., (2015) identified several proteins that share Pfam domains recovered in our analysis, many of which (e.g. cryptochromes, cell adhesion, and regulators of chromatin condensation) have been shown to be involved in photo protection in *Symbiodinium*. Although our current survey does include several Pfam domains corresponding with these proteins (Supplemental Table 3), our analysis did not include any irradiance treatments or gene expression assays. It is worth noting, however, that we did recover a high number of Pfam domains associated with regulators of chromatin condensation (RCC1 & RCC1_2) in *S. muscatinei* and *E. marina* (Fig. 2). A more thorough analysis of the functional domains across various symbiotic associations and levels of irradiance may provide insight into the molecular mechanisms involved in these adaptive traits for dinoflagellates and chlorophytes.

3.4. Thermal tolerance and oxidative stress candidates

We identified several predicted proteins involved with thermal tolerance in *Symbiodinium* in both the *S. muscatinei* and *E. marina* transcriptomes. Among the most abundant thermal- and irradiance-tolerant protein domains in *Symbiodinium* were a variety of different-sized heat shock proteins (HSPs) and proteins involved with the regulation of reactive oxygen species. Collectively, the number of HSPs was significantly greater in *S. muscatinei* than in *E. marina* (Table 3). This greater abundance and diversity is likely linked to the HSPs role as protein chaperones and *Symbiodinium* thermal tolerance (Downs et al., 2013; Hayes and King 1995; Levin et al., 2016; Rosic et al., 2011), but HSPs in these size classes have diverse functions in maintaining cell homeostasis and in intracellular signaling through dynamic interactions with chaperoned “client” proteins. The most numerous HSP protein domain recovered in our analysis resembled HSP-40, with 419 and 30 domains for *S. muscatinei* and *E. marina*, respectively (Table 3). Although the role of HSPs in thermal tolerance in chlorophytes is not thoroughly evaluated, HSP-70 and HSP-40 (or DNAj) proteins have evolved as co-chaperones (Veyel et al., 2014) and as regulators of microtubule stability (Silflow et al., 2011). Although HSP-70 and HP-90 likely play a role in *S. muscatinei* thermal tolerance (Levin et al., 2016), the observed differences among size classes may correspond to different mechanisms that have evolved to increase thermal tolerance. For chlorophytes, HSP-20 may act as a rapid emergency response system capable of immediately binding unfolding proteins in various intercellular components (Kobayashi et al., 2014). Of the thermal-tolerance proteins previously characterized in *Symbiodinium*, the majority of the Pfam results and candidate proteins from the *S. muscatinei* transcriptome resembled previously published analyses (Aranda et al., 2016; Levin et al., 2016). Future investigations involving temperature manipulations and changes in gene expression would permit the identification and characterization of which HSPs are candidates for mediating temperature stress responses in *S. muscatinei* and *E. marina*.

While cellular mechanisms that contribute to bleaching remain incompletely understood, an overall increase in reactive oxygen species (ROS) in response to elevated temperature and light is considered a key factor (Downs et al., 2002; Weis 2008). Direct evidence of ROS production by cnidarian symbionts was first documented in *S. muscatinei*

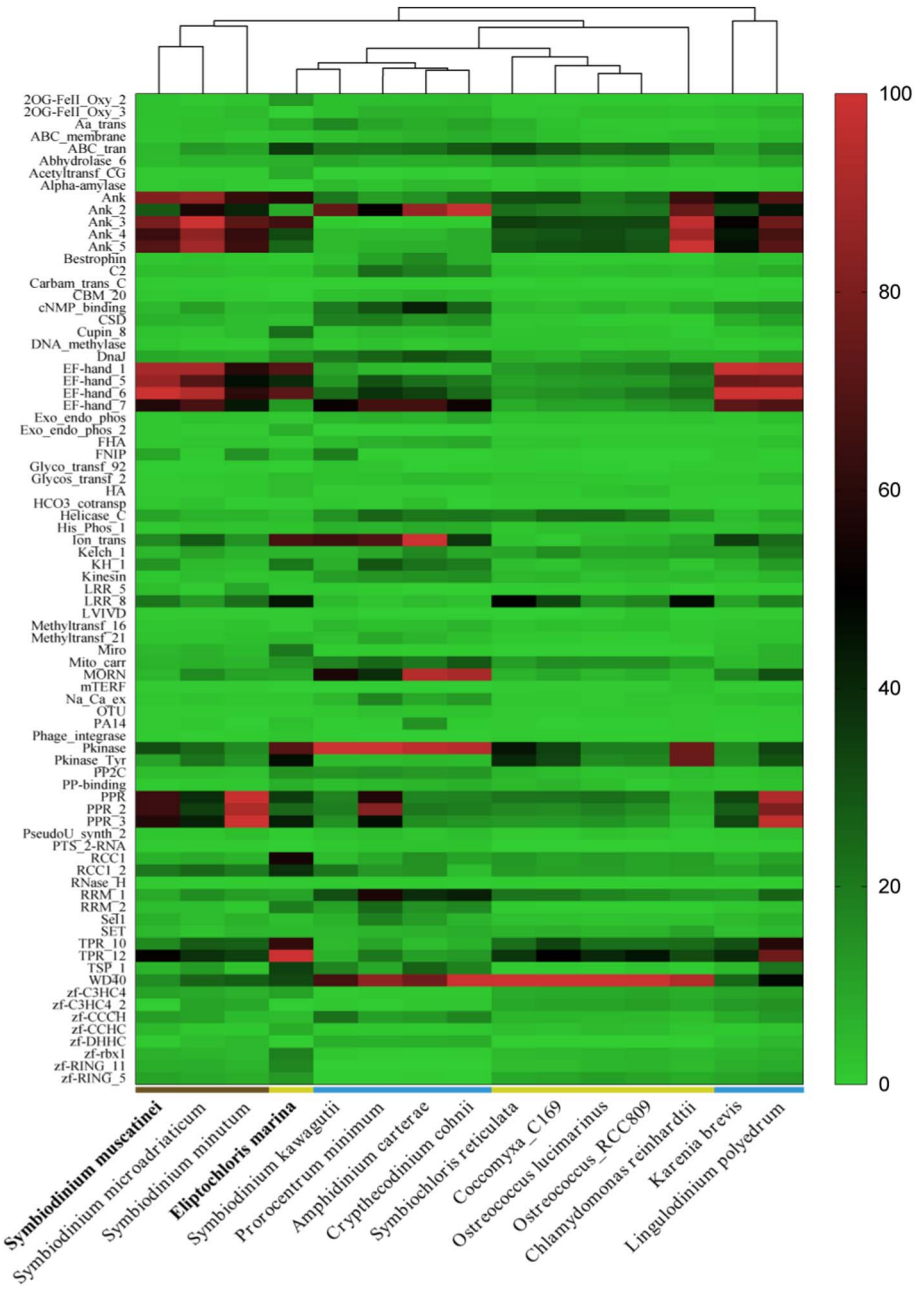

Fig. 2. Heatmap depicting differential Pfam abundance across symbiotic (brown bars) and free-living dinoflagellates (blue bars) and chlorophytes (yellow bars), with the focal taxa noted in bold. Rows shown here indicate the Pfam domains (N = 81) that are significantly different across *Symbiodinium* (see Aranda et al., 2016). Domain counts were normalized for each species, with colors representing proportion of Pfam domains identified. The clustering tree was determined using Pearson correlation coefficients of Pfam domain counts for each species using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, California, USA). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

associated with *A. elegantissima* (Dykens et al., 1992). More recent studies have focused on comparative thermal tolerance and ROS production among diverse *Symbiodinium* species (Levin et al., 2016). Despite these efforts, the molecular control and interspecific variation of ROS production in *Symbiodinium* is not fully understood (Goyen et al., 2017; Rosic et al., 2010). Our Pfam analysis identified protein domains in both *S. muscatinei* and *E. marina* associated with various superoxide dismutases, peroxidases, peroxiredoxins, and cytochrome P450s (Table 3). Dimond et al., (2017) showed that *S. muscatinei* produce slightly more H_2O_2 than *E. marina* on a cell volume-specific basis, which may relate to peroxidases and three of the four superoxide dismutase identified in *S. muscatinei* (Table 3).

The iron superoxide dismutase (Sod_Fe_N) was the only superoxide dismutase Pfam domain found in *E. marina*. Levin et al., (2016) showed that Fe-Sod genes in *Symbiodinium* (type C1) were upregulated when a thermally-tolerant population was exposed to elevated temperatures. This did not seem to happen in thermally-sensitive populations. Top hits during our reciprocal blast searches for *E. marina* and *S. muscatinei* recovered sequences associated with chlorophytes and dinoflagellates, respectively. Beyond these taxonomically-relevant BLAST search results, both *E. marina* and *S. muscatinei* shared high sequence similarity to iron superoxide dismutase genes from bacteria, reinforcing the possibility for horizontal gene transfer or convergence as discussed previously (Krueger et al., 2015) or simply highlighting incomplete sampling in underrepresented lineages.

3.5. Metabolic exchange candidates

We identified several candidate genes and protein domains in the transcriptomes of *S. muscatinei* and *E. marina* hypothesized to be involved in the translocation of nutrients and ions, with notable differences between the two symbionts. Dinoflagellates, including species that are not endosymbionts, have a high number of transmembrane transporters (Aranda et al., 2016; Gómez 2012; Lin et al., 2015). Although possible roles of bicarbonate and ammonium transporter domains in *Symbiodinium* nutrient exchange have been previously

Table 3
Stress response and metabolism Pfam domain count.

Stress response	Pfam domain	*E. marina*	*S. muscatinei*
Heat shock proteins	HSP9_HSP12	0	48
	HSP20	3	17
	HSP33	0	6
	HSP-40/DnaJ	30	419
	HSP70	2	33
	HSP90	2	11
Reactive oxygen species	Cytochrome_CBB3	2	23
	Sod_Cu	0	7
	Sod_Fe_C	0	2
	Sod_Fe_N	1	0
	Sod_Ni	0	6
	Peroxidase	5	10
	1-cysPrx_C	2	5
	p450	2	6
Metabolism			
Transporters	HCO33_cotransp	1	54
	PRO_Ca	0	8
	Ammonium_transp	8	88
Photosynthesis	PCP	3	24

suggested (Aranda et al., 2016; Lin et al., 2015), this is the first exploration into these and similar transporters in *Elliptochloris* and *S. muscatinei*. For *S. muscatinei*, the Pfam domain search identified 62 bicarbonate (HCO_3 & PRO_Ca) and 88 ammonium (Ammonium_transp) transporter domains. This was in sharp contrast to *E. marina*, which only had 1 bicarbonate and 8 ammonium transporter domains in our search (Table 3). The exchange of organic compounds has been studied in *Hydra* and their green algal symbionts (Cook 1972; Muscatine and Lenhoff 1965), but the molecular mechanisms behind this exchange of nutrients remain unknown. The reduced number of bicarbonate and ammonium transporters identified in *E. marina* may contribute to the reduced nutritional input from this symbiont, in addition to other environmental, physiological, and molecular components not characterized in our study (Bingham et al., 2014; Dimond et al., 2013; Verde and McCloskey 1996b, 2002, 2007).

In addition to nutrient and ion transporters, we screened our transcriptomes for light-harvesting proteins, specifically the peridinin-chl-proteins (PCP). The PCP, a soluble protein complex located in the periphery of the thylakoid membrane, has a key role in photosynthesis in dinoflagellates (Govind et al., 1990; Iglesias-Prieto et al., 1991; Reichman 2002; Reichman et al., 2003; Roman et al., 1988; Weis et al., 2002). Our transcriptome assembly correctly reconstructed several complete and partial PCP transcripts in *S. muscatinei* for both the short (13.7 KB) and long (31.4 KB) form (Table 3). As expected, we did not find any PCP transcript in *E. marina*, as these appear to be unique to dinoflagellates (Govind et al., 1990; Jansson et al., 1999). Although the short form was almost identical to what was previously reported (Weis et al., 2002), a larger protein recovered in our analysis has not been isolated previously in *S. muscatinei* and shares a higher sequence identity to the dinoflagellate *Amphidinium carterae* than to other *Symbiodinium* species (Supplemental Table 5). Within the dinoflagellate genome, several conserved PCP gene copies (Le et al., 1997; Reichman et al., 2003) are routinely referred to as being short or long form in *Symbiodinium* (Hiller et al., 2001; Iglesias-Prieto et al., 1991; Reichman et al., 2003; Sharples et al., 1996; Weis et al., 2002). By searching NCBI's TSA dataset, we were able to identify several long-form "High Salt PCP" representatives from additional *Symbiodinium* strains. In our PCP gene tree reconstruction, *S. muscatinei* was missing the traditional long-form group of PCP genes (Fig. 3), instead grouping with a high-salt PCP (HSPCP) group that was previously identified in *A. carterae* (Sharples et al., 1996). This distinct long-form PCP divides the *Symbiodinium*-associated PCP genes into potentially three distinct groups (Fig. 3), which may also reflect functional differences in *S. muscatinei*.

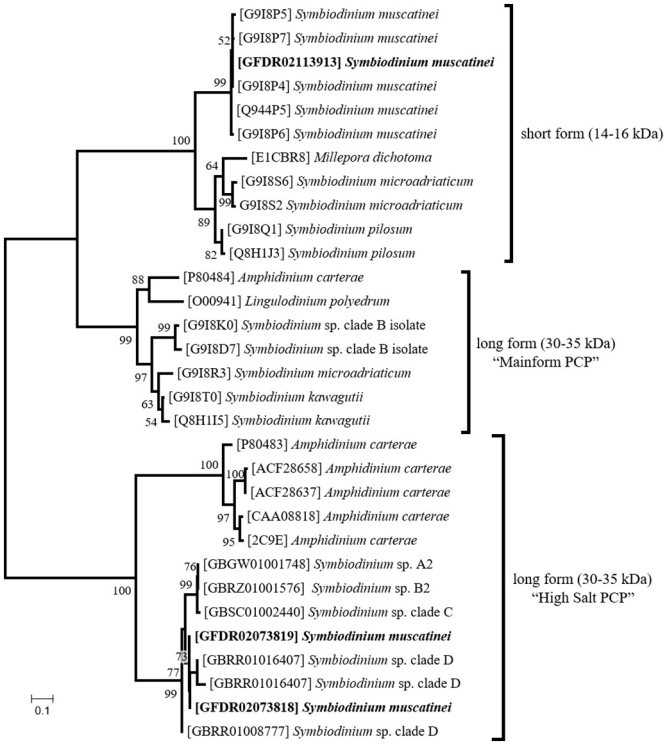

Fig. 3. Maximum Likelihood PCP gene tree produced in FastTree2. Bootstrap support values > 50 are shown. Bold labels indicate new *S. muscatinei* PCP genes identified in this study.

These results indicate that both the short and long forms may be active and contribute to the overall photosynthetic activity of *S. muscatinei*.

4. Conclusions

We used a comparative transcriptomic approach to evaluate transcriptome assemblies of two symbionts commonly associated with the sea anemone *A. elegantissima*. Each symbiont transcriptome exhibits some host contamination, but this appears to be minimal based on our preliminary screening. This dataset provides a necessary reference for future cnidarian-symbiont research. Species-specific genomic repertoires related to thermal tolerance and metabolic exchange provide insight into the molecular diversity present in these symbionts. Although genes in these broad categories are found in both symbionts, the number of protein domains associated with these diverse gene families differs tremendously. Using these data, future investigators should be able to identify genes of interest for either symbiont and test hypotheses regarding host–symbiont interactions under variable environmental and physiological conditions. Future work involving these temperate symbionts will not only aid in our understanding of mechanisms that lead to symbiosis breakdown, but will also help identify molecular mechanisms responsible for symbiont establishment and regulation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.margen.2017.08.010.

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