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Chapter 1

PROTEINS RESPONSIVE TO VARIABLE TEMPERATURE EXPOSURE IN THE REEF-BUILDING CORAL *SERIATOPORA HYSTRIX*

Anderson B. Mayfield[1,2,3,*], Yi-Jyun Chen[2,3], Chi-Yu Lu[4,5] and Chii-Shiarng Chen[1,2,6,7]

[1]Living Oceans Foundation, Annapolis, MD, US
[2]National Museum of Marine Biology and Aquarium, Checheng, Pingtung, Taiwan, R.O.C.
[3]Taiwan Coral Research Center, Checheng, Pingtung, Taiwan, R.O.C.
[4]Department of Biochemistry, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.
[5]Center for Research Resources and Development, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.
[6]Graduate Institute of Marine Biotechnology, National Dong-Hwa University, Checheng, Pingtung, Taiwan, R.O.C.
[7]Department of Marine Biotechnology and Resources, National Sun Yat-Sen University, Kaohsiung, Taiwan, R.O.C.

[*] Author to whom correspondence should be addressed email: andersonblairmayfield@gmail.com; Tel.: + 1-337-501-1976; Fax: +886-2-265-18660.

ABSTRACT

Although reef-building corals engaged in mutualistic relationships with dinoflagellates of the genus *Symbiodinium* are threatened by global climate change, many anthozoan-dinoflagellate endosymbioses display a marked capacity for acclimation with respect to temperature changes. For instance, specimens of the Indo-Pacific reef coral *Seriatopora hystrix* from Southern Taiwan were found to readily acclimate to temperatures that fluctuated from 23 to 29ºC over six hours, a periodicity aimed to simulate local upwelling events that are common during boreal summer spring tides. To gain greater insight into the molecular mechanisms underlying this ability to acclimate to a variable temperature regime, proteins from corals exposed to both stable (26ºC) and variable temperatures for one week were electrophoresed across two dimensions, and differentially expressed proteins were sequenced with mass spectrometry. Seventy-five (64%) and forty-two (36%) proteins were expressed at higher levels by coral hosts and their *Symbiodinium* populations, respectively, of the stable temperature treatment. This suggests that a number of cellular pathways, including lipid body stabilization and metabolism in the *Symbiodinium* cells, are down-regulated upon exposure to variable temperature, and the potential shift in energy modulation implied by these findings may play a role in the restoration of homeostasis necessitated by exposure to such highly variable temperature conditions.

INTRODUCTION

Most current global climate change (GCC) models assume that reef-building corals are unable to acclimate to changes in their abiotic environment [1]. Although it is true that many corals are known to live near the upper threshold of their thermotolerance and readily bleach in response to sustained temperature increases [2-3], recent studies have revealed that not only can corals readily acclimate to elevated temperature, salinity, and pCO_2 [4-7], but they can thrive under such conditions [8-10]. For instance, corals from Houbihu, Taiwan (Figure 1A) are readily exposed to episodic, spring tide upwelling during the boreal summer, periods during which temperatures may change up to 9-10ºC within several hours [11]. Corals from these upwelling habitats have proven to be markedly resilient to both short- [12] and long-term [13] increases in temperature, as has been predicted to occur based on studies of intertidal organisms [14].

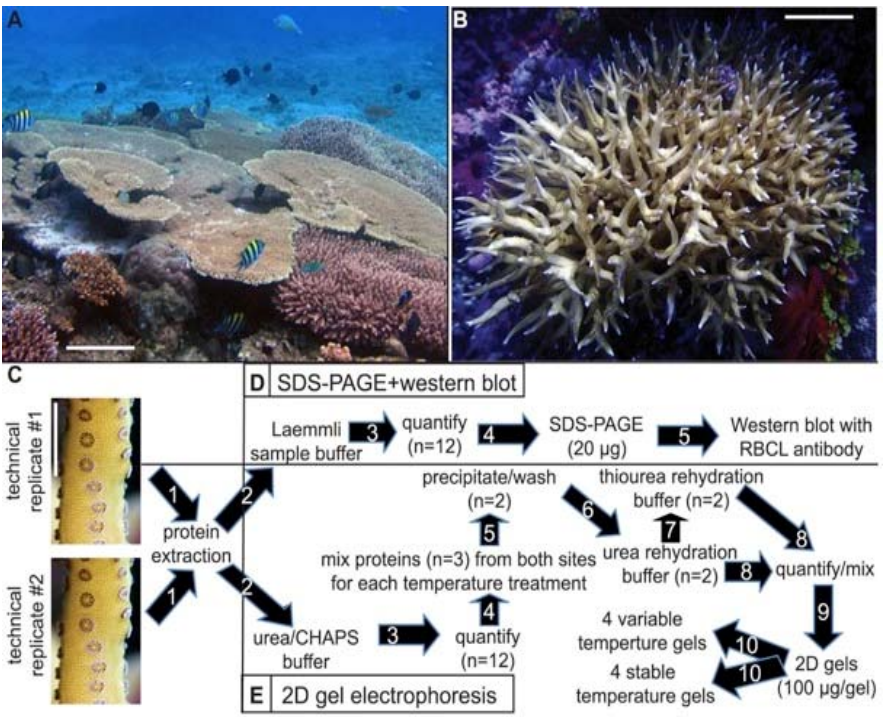

Figure 1. The upwelling field site Houbihu, the model coral *Seriatopora hystrix*, and an analytical flow-chart of the proteomic analyses. (A) Houbihu, the upwelling site from which half of the *Seriatopora hystrix* specimens used in the variable temperature study were sampled (photograph taken by Dr. Pi-Jen Liu, National Museum of Marine Biology and Aquarium, Taiwan). (B) An adult *S. hystrix* colony. (C) Proteins were extracted from each of two technical replicates (i.e., nubbins) from each of the 12 experimental aquaria after a 7-d exposure to either variable (23-29ºC over a 6-hr period; n = 6 aquaria) or stable temperature (26ºC; n = 6 aquaria). (D) The expression of RBCL was quantified in the 12 samples dissolved in SDS-PAGE sample buffer. (E) For the second technical replicate from each aquarium, proteins were prepared for 2-dimensional gel electrophoresis as described in the text. Proteins were pooled across sites of origin (SO; i.e., proteins from corals from Houbihu were mixed with those of corals from Houwan) for each of the two temperature treatments (TT) given the fact that *only* a TT effect on protein expression was of interest herein. The numbers on the arrows represent the respective experimental steps in E, and the scale bars in panels A, B, and C represent 500, 50, and 5 mm, respectively.

Table 1. Summary of results of the Seriatopora hystrix variable temperature study (SHVTS). Site of origin (SO), temperature treatment (TT), and interaction effects were deemed statistically significant at $\alpha < 0.05$ (denoted by "*" in the respective cells). "Upwelling site" and "non-upwelling site" refer to Houbihu and Houwan, respectively. NA = not applicable. Chl-a = chlorophyll a

Response variable	SO effect	TT effect	Inter-action effect	Major finding(s)	Refer-ence
Host coral genotype		NA	NA		[17]
Symbiodinium genotype					[17]
Growth			*	Faster growth in non-"transplanted" corals	[15]
Symbiodinium density	*			Non-upwelling site > upwelling site	[15]
Chl-a concentration		*	*	Variable temperature > stable temperature Higher chl-a in non-"transplanted" corals	[15]
Maximum dark-adapted quantum yield of photosystem II (Fv/Fm)	*	*	*	Upwelling site > non-upwelling site Variable temperature > stable temperature Higher FV/FM in non-"transplanted" corals	[15]
Symbiodinium heat shock protein 70 (hsp70) genome copy proportion (DNA content)					[15]
RNA/DNA ratio					[15]
Protein/DNA ratio	*			Non-upwelling site > upwelling site	[15]
Symbiodinium ribulose-1,5-bisphosphate carboxylase/oxygenase (rbcL) mRNA expression		*		Variable temperature > stable temperature	[15]
Symbiodinium photosystem I (subunit III; psI) mRNA expression	*	*		Upwelling site > non-upwelling site Variable temperature > stable temperature	[15]
Symbiodinium phosphoglycolate		*		Variable temperature > stable temperature	[15]

Response variable	SO effect	TT effect	Inter-action effect	Major finding(s)	Refer-ence
phosphatase (pgpase) mRNA expression					
Symbiodinium ascorbate peroxidase (apx1) mRNA expression					[15]
Symbiodinium hsp70 mRNA expression		*		Variable temperature > stable temperature	[17]
Symbiodinium nitrate transporter 2 (nrt2) mRNA expression					[17]
S. hystrix hsp70 mRNA expression		*		Stable temperature > variable temperature	[17]
S. hystrix α-tubulin (tuba) mRNA expression					[17]
S. hystrix tropomyosin (trp1) mRNA expression					[17]
S. hystrix β-actin (actb) mRNA expression		*		Variable temperature > stable temperature	[17]
S. hystrix ezrin mRNA expression					[17]
S. hystrix phospholipase α-2 (cplap2) mRNA expression			*	Higher mRNA expression in "transplanted" corals	[17]
S. hystrix transient receptor cation channel (trcc) mRNA expression					[17]
S. hystrix organic anion transporter (oatp) mRNA expression					[17]
Symbiodinium RBCL protein expression					herein
Protein expression (2D gel)		*		Stable temperature > variable temperature	herein

Table 2. A breakdown of the 10 sequenced protein spots by compartment of origin: coral host or *Symbiodinium*. Of the 117 unique peptides that were sequenced and met the minimal inclusion threshold criteria (described in the main text), 75 (64%) were from the coral host, *Seriatopora hystrix*, and 42 (36%) were from the dinoflagellate endosymbionts (genus *Symbiodinium*) living within the hosts' gastrodermal cells. Two 2-sample proportion tests were conducted to determine if one compartment (host coral or *Symbiodinium*) was over-represented in the partially sequenced proteome within each spot; for the first test, the raw proportions were compared (non-adjusted). For the second, the total number of *Symbiodinium* proteins was multiplied by 1.8 to adjust for the fact that the host contributed 75 of the 117 unique proteins (i.e., 64% host/36% *Symbiodinium* = 1.8) across all 10 spots. For the "Total/Average" row, the total number of proteins is given for the 3rd-5th columns while the average percentages are given for the "% host" and "% *Symbiodinium*" columns; error terms represent standard deviation for the latter. NS = not significant (2-sample proportion test, $p > 0.05$). NA = not applicable. kDa = kilodalton. pI = isoelectric point

Spot	Molecular weight (kDa)	pI	# host proteins	# *Symbiodinium* proteins	# total proteins	% host	% *Symbiodinium*	2-sample proportion test *p* (non-adjusted)	2-sample proportion test *p* (adjusted)	Conclusion
1	27.4	4.9	6	7	13	46	54	NS	NS	
2	27.3	5.0	12	7	19	63	37	NS	NS	
3	27.4	5.2	14	4	18	78	22	<0.001	<0.05	Host > *Symbiodinium*
4	27.3	5.5	13	4	17	77	23	<0.01	NS	
5	20.8	4.8	16	11	27	59	41	NS	NS	
6	21.1	5.1	6	8	14	43	57	NS	<0.05	*Symbiodinium* > host
7	20.9	5.3	8	6	14	57	43	NS	NS	
8	20.7	5.3	10	8	18	56	44	NS	NS	
9	20.4	5.5	9	5	14	64	36	NS	NS	
10	20.0	5.9	6	8	14	47	53	NS	<0.05	*Symbiodinium* > host
Total/Average			100	68	168	59 ± 12	41 ± 12	NA	<0.0001	Host > *Symbiodinium*
Unique			**75**	**42**	**117**	**64**	**36**	NA	<0.0001	Host > *Symbiodinium*

In order to gain insight into how corals from these upwelling sites acclimate to such dramatic temperature changes, an experiment was conducted in which corals from not only Houbihu, but also a nearby, non-upwelling site, Houwan, were exposed to either a variable (23-29ºC over a 6-hr period) or stable (26ºC) temperature profile for seven days [15-17]. *Seriatopora hystrix* (Figure 1B-C) was chosen as the model coral for such laboratory-based studies, given its 1) widespread distribution across the Indo-Pacific [18-19], 2) propensity for bleaching under periods of elevated temperatures [20], and 3) modest existing understanding of its molecular eco-physiology [21-22]. In general, even *S. hystrix* specimens that were never exposed to upwelling *in situ* readily acclimated to variable temperature conditions (Table 1), and an effort was made to develop both a physiological and a sub-cellular understanding of how such acclimation occurred in the samples from this "*Seriatopora hystrix* variable temperature study" (SHVTS; [15-17]).

Given recent success in employing molecular biology-driven approaches to answering an array of both fundamental [23-27] and stress/environmental biology [28] questions in the field of anthozoan-dinoflagellate endosymbiosis, the expression of a series of gene mRNAs was measured in samples of the SHVTS [15, 17]. Although several genes encoding proteins involved in photosynthesis were differentially expressed between the stable and variable temperature treatments (TT; [15, 17] and Table 1), the variation was generally modest, and it was, furthermore, unclear whether such changes in mRNA expression would actually lead to altered levels of translation of the respective proteins; indeed, in the few studies that have looked at both gene and protein expression in the same anthozoan-dinoflagellate sample [7, 26], there was not always a significant, positive correlation between gene and protein expression [7]. Therefore, a whole-proteome-based approach employing two-dimensional (2D) electrophoresis followed by protein sequencing via mass spectrometry (MS) was taken herein in order to better unravel the molecular means by which *S. hystrix* and its endosymbiotic *Symbiodinium* populations acclimate to a variable temperature regime.

MATERIALS AND METHODS

SHVTS

The SHVTS was discussed in previous works [15-17]. Briefly, six *S. hystrix* colonies from both the upwelling (Houbihu; Figure 1A) and non-upwelling (control) sites (Houwan) were collected, acclimated in indoor

aquaria to allow for recovery from transplantation [16], fragmented into nubbins, acclimated again for several weeks, and randomly assigned to 1 of 12 experimental aquaria: 3 for each site of origin (SO) x TT combination. For protein work, ~100 mg pieces/branches from each nubbin (n = 2 pseudo-/technical replicates per aquarium) were immediately immersed in TRIzol® (Life Technologies, USA) after a 7-d exposure to either a stable (26ºC, n = 6 aquaria) or variable TT (23-29ºC over a 6-hr period, n = 6 aquaria), with time 0 samples (~100 mg; n = 2 pseudo-replicated nubbins/aquarium) taken just before the temperature began to fluctuate in the variable temperature aquaria; such samples (n = 6 for each SO) were collected to uncover SO, rather than TT (the focus of this work), differences and are not discussed further herein. Samples were frozen in TRIzol at -20ºC until the day of extraction. The remainder of each of the 48 nubbins (2 nubbins/aquarium x 12 aquaria [3 for each of 4 SO x TT interaction groups] x 2 sampling times [t = 0 and 7 d]) was used for a variety of additional molecular and physiological analyses discussed in Table 1 and in prior works [15-17]. RNAs and DNAs were isolated from the same 100-mg fragments from which the proteins, discussed below, were isolated, as TRIzol permits the extraction of high quality RNA, DNA, and protein from the same biological sample [12].

Protein Extraction

Proteins were extracted from ~100-mg fragments from each of the 24 nubbins sampled at the t = 7 d sampling time (2 pseudo-replicates/aquarium x 12 aquaria) with TRIzol as recommended by the manufacturer except with 10-min sonications on ice between the washes with "protein wash I." As mentioned above, the respective RNAs and DNAs from each of the same 24 samples were already purified and analyzed [15-17]. For one of the two technical replicates from each aquarium (Figure 1C), the proteins were dissolved in 100-200 µl Laemmli sample buffer [29] without the additional of bromophenol blue (Figure 1D), boiled, spun at 12,000 *xg* for 10 min at 4ºC, and the supernatants were transferred to a new 1.5-ml microcentrifuge tube. Approximately 20-25 µl of protein were quantified with the 2D Quant kit (Amersham Biosciences, USA), as recommended by the manufacturer. For the second of the two technical replicates from each aquarium (Figure 1E), the proteins were purified as described above except they were dissolved in rehydration buffer (8 M urea, 2% CHAPS; i.e., "urea/CHAPS buffer" of Figure 1E) at room temperature (RT) for 2-3 hr, with constant, vigorous

agitation. Approximately 20-25 µl of protein were quantified as described above, and proteins were frozen at -80ºC.

Because the temperature regime itself, rather than the SO, was found to have a greater influence on coral physiology based on previous analyses (Table 1), proteins to be electrophoresed across two dimensions were pooled across SO. However, given the oceanographic differences between Houbihu and Houwan, future work should seek to look at SO differences in addition to TT alone, as was done herein, in order to uncover how environmental history drives the future physiological response to altered abiotic conditions. After several days of storage at -80ºC, proteins dissolved in the initial urea/CHAPs buffer were thawed, and three samples (one from each aquarium) from each of the two SO from the same TT (n = 6 protein aliquots/TT) were mixed in equimolar concentrations:

Sample 1: 3 Houwan-stable TT samples + 3 Houbihu-stable TT samples = *1 stable TT* sample pooled across SO to be analyzed by 2D + MS.

Sample 2: 3 Houwan-variable TT samples + 3 Houbihu-variable TT samples = *1 variable TT* sample pooled across SO to be analyzed by 2D + MS.

These two, pooled protein samples were precipitated with 2 ml acetone supplemented with 0.07% beta-mercaptoethanol (BME) at -80ºC for 1 hr. Protein pellets from the stable and variable TT samples were washed thrice with acetone-BME, dried on the benchtop at RT, and dissolved in 150 µl of the rehydration buffer recommended by Jacobs et al. [30]: 9.5 M urea, 2% CHAPS, 0.5% carrier ampholytes (GE Healthcare, USA), and 65 mM dithiothreitol (DTT). Samples in this "urea rehydration buffer" (Figure 1E) were vortexed vigorously for several minutes, spun at 12,000 *xg* for 10 min at 4ºC, and the supernatants were transferred to new tubes and quantified (20-25 µl aliquots) as described above.

The remaining, un-solubilized protein pellets were dissolved in 150 µl of the "thiourea rehydration buffer" (Figure 1E) described by Jacobs et al. [30]: 2 M thiourea, 7 M urea, 4% CHAPS, 0.5% carrier ampholytes, and 65 mM DTT. In general, this allowed the remaining proteins to be solubilized. Then, 20-25 µl of these proteins were quantified as described above. Because preliminary experiments found that urea and thiourea rehydration buffer-dissolved proteins presented different profiles on 2D gels (data not shown), approximately 200 µg protein from each TT and solubilization buffer were mixed to yield 400 µg protein in urea + thiourea buffer for each of the two TT,

a sufficient quantity for running 4 100-µg 2D gels (i.e., four technical replicates/sample) for each of the two pooled protein samples.

2D Gel Electrophoresis 1st Dimension-Isoelectric Focusing

Isoelectric focusing (IEF) was used for the first dimension of the 2D gel with the Ettan IPGphor IEF system (Amersham Biosciences). Four gels were run for each of the two TT: stable and variable, and approximately 100 µg protein were loaded into each of the eight gels. Samples, which represented a mix of proteins from samples of both SO, as well as a mix of both urea and thiourea-based buffers, were diluted to 125 µl with the addition of thiourea buffer and, if necessary, additional carrier ampholytes to where the latter was at a final concentration of 0.5%. Proteins of each of the two TT were focused at the same time on different IEF strips (i.e., one of the four stable TT protein samples was run at the same time as one of the four variable TT protein samples). Along the center of the bottom of the IEF strip holder, proteins (100 µg/TT; 125 µl) were loaded evenly from left to right, while simultaneously ensuring that there were no air bubbles. The protective membrane was removed from the IEF strip (pH 4-7, 7 cm, Amersham Biosciences), which was then placed into the strip holder with the gel side down. Then, 200 µl of dry strip cover fluid were aliquoted over the strip, and the lid was placed over the strip holder. The two strip holder units (one for each of the two co-run samples) were placed in the Ettan IPGphor IEF electrophoresis chamber (Amersham Biosciences), and the following program was run at 20ºC: 50 V for 12 hr (rehydration), 300 V for 60 V-hr along a gradient, 600 V for 120 V-hr along a gradient, 1000 V for 500 V-hr along a gradient, 2000 V for 1000 V-hr along a gradient, 5000 V for 6000 V-hr, and 50 V for 10 hr. The same protocol was used on the three additional pairs of stable and variable TT samples, which were electrophoresed on different days.

2D Gel Electrophoresis 2nd Dimension-SDS-PAGE

Chromatography paper (Whatman, USA) was cut to a 1 x 0.5 cm size and overlaid with 5 µl protein marker (Fermentas PageRuler™ prestained protein ladder, Life Technologies). Then, ~ 1 ml of 1% agarose was aliquoted onto a smooth sheet of plastic wrap, and the chromatography paper was placed over the agarose. An additional 1 ml of 1% agarose was then overlaid on the

chromatography paper. After the agarose solidified, the chromatography paper was removed from the agarose to where a 1 mm distance was maintained around the paper. Meanwhile, the IEF strips were immersed in equilibration buffer (6 M urea, 2% SDS, 30% glycerol, 50 mM Tris-HCl [pH 8.8], 0.002% bromophenol blue, and 1% DTT) at RT for 15 min. Then, strips were transferred to the same buffer, except with 1% iodoacetamide (IAA) instead of 1% DTT, for 15 min at RT, washed with SDS-PAGE running buffer to remove residual IAA, and placed on top of a 5-14% stacking-separating Tris-glycine SDS-PAGE gel. Electrophoresis was conducted on ice at 70 V for ~30 min and 120 V for 1-2 hr in a Mini-PROTEAN® Tetra cell (BioRad, USA), with two samples (one stable and one variable) run at the same time. In total, eight 2D gels were run (four technical replicates for each of two TT), though only two gels were run at any given time (i.e., four days were required to run all eight gels).

Each of the eight gels was fixed in 50% methanol and 7% acetic acid for 30 min after removing the stacking gel. Then, the gels were stained with SYPRO® Ruby (Life Technologies) on a shaker table in the dark overnight. The gels were then destained in 10% methanol and 7% acetic acid for 30 min and imaged with a Typhoon Trio™ scanner (GE Healthcare) at 312 nm (aperture = 2.8, exposure time = 2.4 s). In general, there were no unique protein spots between the stable and variable TT (Figure 2A-B), though this could be due to the low amount of protein loaded (100 µg per gel). Since *Symbiodinium* density was similar between TT (Table 1), the protein spot intensity values of each of the eight gels (data not shown) were *not* normalized to a genome copy proportion (GCP) prior to the subtraction step (described below). In contrast, the target protein (RBCL) data, described below, warranted the use of a GCP given that both SO and TT effects were tested in that analysis, and a difference in *Symbiodinium* density between SO was documented previously (Table 1 and [15]).

Image analysis software (ImageQuantTL) provided with the scanner was used to perform a "subtraction" whereby the gel image of one stable TT replicate sample was overlaid on the variable TT replicate run and processed simultaneously to better portray differentially expressed proteins (Figure 2C-D). This subtraction was performed on the four pairs of stable vs. variable TT run on four different days. Because no unique spots were evident in any of the four pairs of gels, proteins found to be expressed at significantly higher quantities by ImageQuantTL in the stable temperature gel were instead targeted, and 10 protein spots that were found to be over-expressed in all four stable TT gels (i.e., all technical replicates; Figure 2C) relative to their variable

temperature counterparts were excised from a representative stable TT gel (Figure 2D) with sterilized 200-µl pipet tips and placed into 1.5-ml microcentrifuge tubes.

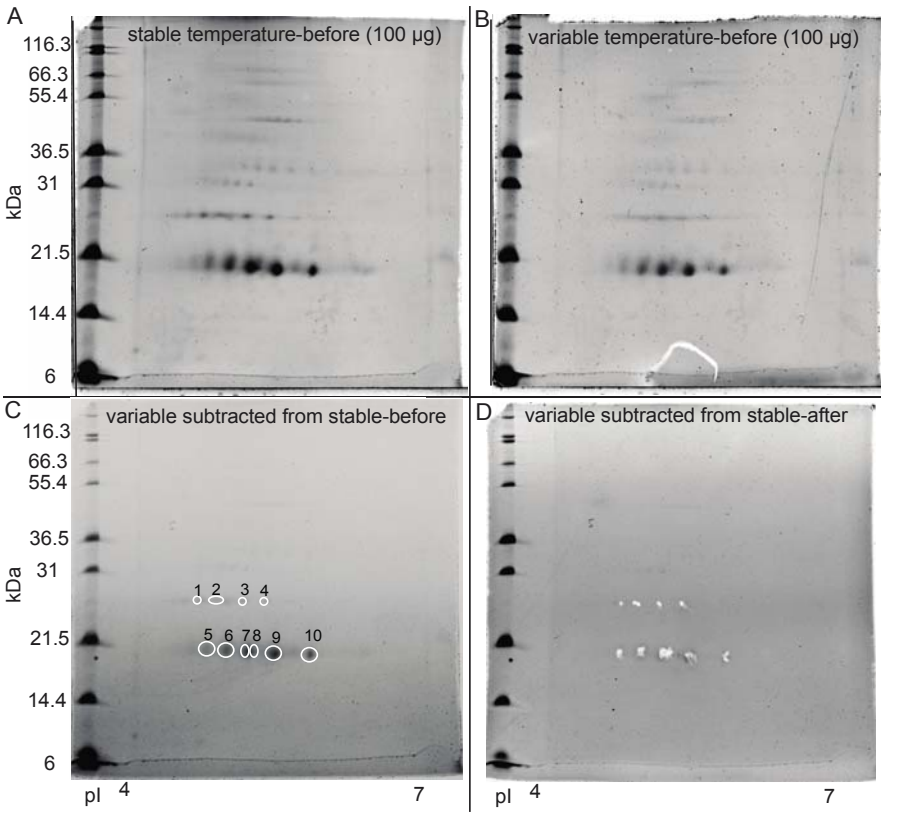

Figure 2. 2-dimensional gel electrophoresis of proteins expressed by *Seriatopora hystrix* specimens exposed to either a variable or stable temperature regime for seven days. kDa = kilodalton. pI = isoelectric point.

The ten excised protein spots (Table 2) were destained and in-gel digested as follows. First, the protein + gel slabs were washed in 50% acetonitrile in 25 mM ammonium bicarbonate (pH 8.5). Then, they were incubated in 100 µl of the same acetonitrile solution for 15 min and spun at 10,000 *xg* for 1 min. The supernatant was removed and replaced with 100 µl of 100% acetonitrile, and the samples were incubated for 5 min. The samples were spun again as above, and the supernatant was removed. The gel bits were allowed to dry for 5 min before incubation with 30 µl trypsin (a 2 µg aliquot that had been re-suspended

in 1 ml water and 1 ml 50 mM ammonium bicarbonate) at 37ºC overnight. The next day, samples were centrifuged at 10,000 *xg* for 1 min, and the supernatant was transferred to a new microcentrifuge tube. Then, 50 µl of 50% acetonitrile and 5% trifluoroacetic acid (TFA) were added to the remaining samples, which were then sonicated 10 times (10 s each time). Samples were centrifuged again at 10,000 *xg* for 1 min, and the supernatant was combined with the supernatant from the first spin. Another round of 50% acetonitrile/5% TFA incubation followed by sonication/spinning/supernatant collection was conducted, and the third supernatant was combined with the previous two. The supernatant was dried for 1-2 hr prior to shipping to the MS facility at Kaohsiung Medical University's (KMU) Center for Research Resources and Development's Core Proteomics facility, where the 10 protein spots were analyzed by MS, as described below.

MS

After trypsin digestion, 2 µl of the digested peptides were injected into the nano-liquid chromatography (LC) system and detected by an LTQ Orbitrap Discovery Hybrid Fourier Transform Mass Spectrometer (FTMS; Thermo-Fisher, USA) at a resolution of 30,000 coupled with a nanospray source that was executed in the positive ion mode. The Nano-UPLC system (nanoACQUITY UPLC) was purchased from Waters (USA), as were the desalting (Symmetry C18, 5 µm x 180 µm x 20 mm) and analytical (BEH C18, 1.7 µm x 75 µm x 150 mm) columns. The peptide eluate from the column was directed to the nanospray source, and the MS was operated in positive ion, data-dependent mode.

MS Data Analysis

Raw data files (mascot generic format [.mgf]) were processed with Mascot distiller software (version 2.2, Matrix Science, USA) and then uploaded onto the Mascot server hosted by KMU. Several Mascot protein databases (Tables 3-4) were queried using Mascot's default search parameters. Comparison of MS data against NCBI's nr database via Mascot yielded mainly common protein contaminants (human keratins, actins, etc.). However, upon comparing spectral data against the *Hydra magnipapillata* and *Acropora digitifera* (coral) proteomes, as well as a suite of others, a variety of both host coral and *Symbiodinium* peptides were identified. The *A. digitifera* proteome was

conceptually translated previously and converted into a Mascot searchable database by Li et al. [31] and is referred to as "NMMBA" in Tables 3-4.

One of two criteria was required to have been met to determine verification of "presence" of a protein: either 1) 15 consecutive amino acid (AA) residues were sequenced or 2) two unique peptides mapping to the same protein were sequenced, and the total length of both peptides was 15 AA or more. After using Mascot to determine the likely identity of each protein, individual peptide sequences were BLASTed (BLASTp) against the NCBI database to further verify the identities of the sequenced proteins. Proteins fulfilling the minimum criteria established *a priori* were assigned a functional category from the "Pfam" database, and 2-sample proportion tests were used to determine whether proportional differences existed between compartments (coral vs. *Symbiodinium*) in the functional categories in which the identified proteins were grouped. Two-sample proportion tests were also used to determine whether was compartment was over-represented in each of the 10 spots (Table 2). Bacterial proteins were excluded from analysis, though should be more carefully considered in future works given the importance of probiotic microbes in maintaining coral health.

Western Blotting

Given that significant differences in ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene expression were documented across TT in the SHVTS [15], the respective protein, RBCL, was targeted herein for expression analysis with western blotting with a commercially available antibody from Agrisera (Sweden). Proteins (20 µg) representing one sample from each of the 12 experimental aquaria (n = 3 for each of the four SO x TT groups), as well as positive controls (30 µg protein from *Pocillopora damicornis* larvae exposed to ambient temperature and pCO_2 from Putnam et al. [7]), were electrophoresed on two 4-10% SDS-PAGE gels as in Mayfield et al. [12]; one SDS-PAGE gel was stained with SYPRO Ruby as described above for staining of the 2D gels and visualized on a Typhoon Trio scanner for assessment of protein quality. Proteins within the second gel were transferred to a polyvinylidene fluoride (PVDF) membrane on ice at 100 V for 75 min. Afterwards, the protein-laden membrane was stained with Ponceau S (Sigma, USA) according to the manufacturer's recommendations in order to visualize degree of protein transfer.

Table 3. Seventy-five host coral proteins expressed at higher levels in specimens of the stable temperature treatment in the 2010 *Seriatopora hystrix* variable temperature study. For proteins found in multiple spots, the range of molecular weights (in kilodaltons [kDa]) and isoelectric points (pI) have been provided. The average total length of all peptides mapping to a unique protein was 43 ± 33 (standard deviation for this and all values henceforth) amino acids (AA), and ranged from 15 (the *a priori*-set, lower cut-off value) to 212 AA (serine/arginine repetitive matrix protein 2). Coverage (number of sequenced AA/total AA in the hypothesized, full-length protein x 100) averaged 7.4 ± 8.2% and ranged from 1 to 43%. Please see Table S1 for the associated peptide sequences.

Protein name	Total length of all peptides	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	Score	NCBI accession	Coverage (%)
Cell surface proteins (n = 4)									
fibrocystin	100	1	678/27.4	8.2/4.9	NMMBA	*Saccoglossus kowalevskii*	19	A7SPV0	1
integrin α	69	7	112/20.9	5.6/5.3	Acropora	*Acropora millepora*	17	ABY74498	6
integrin β2	36	5-6	85.0/20.8-21.1	5.3/4.8-5.1	Acropora	*A. millepora*	14	ABY74499	4
H6 G-protein coupled receptor	19	9	34.7/20.4	9.7/5.5	Acropora	*A. millepora*	15	ABI50929	6
Circadian rhythm (n = 1)									
cryptochrome photolyase (CRY2)	91	3, 6-7, 10	59.9/ 20.0-27.4	8.8/ 5.1-5.9	Acropora	*A. millepora*	16	ABP97099	17
cytoskeleton (n = 2)									
ciliary dynein heavy chain	21	5	525/20.8	5.7/4.8	nr other metazoa	*Pediculus humanus corporis*	38	XP_002426312	1
stathmin	19	5	34.7/20.8	9.8/4.8	nr other metazoa	*Trichoplax adhaerens*	50	XP_002114295	5
Development (n = 2)									
hedgehog	30	2	90.9/27.3	6.4/5.0	NMMBA	*Acropora cervicornis*	41	GASU01035919	43
embryonic polarity protein dorsal	19	7	93.2/20.9	5.4/5.3	nr other metazoa	*Culex quinquefasciatus*	42	XP_001844078	2

Table 3. (Continued)

Protein name	Total length of all peptides	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	Score	NCBI accession	Coverage (%)
DNA binding/transcription factor (n = 8)									
nuclear receptor AmNR2	63	3, 8	49.9/ 20.7-27.4	6.0/ 5.2-5.3	Acropora	*A. millepora*	34	AF323681_1	14
sex comb on midleg-like protein 2	50	7-8	117/20.7-20.9	8.3/5.3	*Hydra magnipapillata*	*H. magnipapillata*	34	XP_002160709	3
nuclear receptor 6	49	1, 2, 10	38.8/ 20-27.4	8.3/ 4.9-5.9	Acropora	*A. millepora*	18	AF323686_1	13
estrogen receptor-β	48	2, 4	59.2/27.3	8.8/ 5.0-5.5	Selenoprotein	*H. sapiens*	29	ESR2_HUMAN	8
topoisomerase (DNA) III β	36	8	153/20.7	8.6/5.3	*H. magnipa-pillata*	*H. magnipapillata*	23	XP_002155556	2
zinc finger protein	26	10	71.3/20.0	9.2/5.9	*H. magnipa-pillata*	*H. magnipapillata*	32	XP_012561483	4
nuclear receptor AmNR8	18	3	42.5/ 27.4	8.8/ 5.2	Acropora	*A. millepora*	16	AF323688_1	4
DM domain protein	17	2, 9	51.5/ 20.4-27.3	9.0/ 5.0-5.5	Acropora	*A. millepora*	16	AF530064_1	3
DNA Repair (n = 1)									
Fanconi anemia group J protein	50	2	125/27.3	6.3/5.0	NMMBA	*Danio rerio*	36	K7GJE0	4
Growth factor (n = 1)									
Imaginal disc growth factor	18	3-4, 9	49.4/ 20.4-27.4	6.7/ 5.2-5.5	nr other metazoa	*Diaprepes abbreviates*	41	AAV68692	4
Glycoprotein (n = 1)									
mucin-17	98	9	574/20.4	5.4/5.5	*H. magnipa-pillata*	*H. magnipapillata*	26	XP_002160582	2
Immunity (n = 1)									
beta-defensin	22	2	11.8/27.3	9.1/5.0	Defensin	*Canis lupus*	26	Q30KS7	22

Protein name	Total length of all peptides	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	Score	NCBI accession	Coverage (%)
Metabolism (n = 8)									
thioredoxin reductase (isoform 1)	54	3, 5	56.2/20.8-27.3	7.2/4.8-5.2	Selenoprotein	*H. sapiens*	14	AF044212_1	10
porphobilinogen deaminase	39	6	39.3/21.1	6.7/5.1	SwissProt	*H. sapiens*	24	HEM3_HUMAN	11
carboxylesterase 1C	37	3	61.0/27.4	5.0/5.2	SwissProt	*Mus musculus*	55	EST1C_MOUSE	7
carbamoyl-phosphate synthetase/aspartate transcarbamylase/ dihydroorotase	29	9	101/20.4	6.8/5.5	*H. magnipapillata*	*H. magnipapillata*	19	NP_001267868	3
alpha-L-arabinofuranosidase B	27	9	35.6/20.4	8.9/5.5	*H. magnipapillata*	*H. magnipapillata*	28	XP_002165874	8
phospholipase D	21	4	104/27.3	8.5/5.5	nr other metazoa	*Schistosoma mansoni*	38	XP_002576773	2
cytochrome 450	19	4	56.9/27.3	9.0/5.5	nr other metazoa	*T. adhaerens*	38	XP_002112306	3
mannosyl-glycoprotein endo-beta-N-acetylglucosamidase[a]	15	1	37.7/27.4	9.3/4.9	*Staphylococcus aureus*	*S. aureus*	23	WP_000247512	4
Mitosis (n = 1)									
inner centromere protein	17	4	112/27.3	9.3/5.5	*H. magnipa-pillata*	*H. magnipapillata*	26	XP_002167890	2
mRNA Processing (n = 15)									

Table 3. (Continued)

Protein name	Total length of all peptides	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	Score	NCBI accession	Coverage (%)
serine/arginine repetitive matrix protein 2	212	5	295/20.8	12/4.8	SRProtein	*M. musculus*	22	SRRM2_MOUSE	7
pre-mRNA-splicing factor CWC22	127	3	65.3/27.4	9.3/5.2	SRProtein	*Bombus impatiens*	36	XP_003494375	22
pre-mRNA splicing factor prp8[b]	67	4	326/27.3	8.4/5.5	SRProtein	*Trichophyton tonsurans*	28	EGE00188	2
pre-mRNA-splicing factor CDC5/ CEF1[b]	55	5	87.0/20.8	6.5/4.8	SRProtein	*Pseudogymnoascus destructans*	22	ELR07040	7
transformer-2 protein homolog beta-like	52	7	82.2/20.9	11.6/5.3	TRA2-beta	*Salpingoeca rosetta*	14	EGD83325	7
pre-mRNA-splicing ATP-dependent RNA helicase prp28[b]	51	5	88.3/20.8	8.8/4.8	SRProtein	*Neosartorya fischeri*	35	PRP28_NEOFI	6
ATP-dependent RNA helicase DHX8-like	51	3	137/27.4	8.5/5.2	SRProtein	*S. kowalevskii*	22	XP_002734274	4
CWC25 spliceosome-associated protein homolog	42	5	58.1/20.8	10.0/4.8	SRProtein	*S. kowalevskii*	17	XP_002732008	8
pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16	37	5	144/20.8	6.4/4.8	SRProtein	*Oryzias latipes*	27	XP_004067034	3

Protein name	Total length of all peptides	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	Score	NCBI accession	Coverage (%)
pre-mRNA-splicing factor SYF1[b]	32	4	41.8/27.3	5.5/5.5	SRProtein	*Rhizoctonia solani*	31	CCO31421	8
KH domain containing, RNA-binding, signal transduction-associated 3	29	5	38.3/20.8	6.5/4.8	*H. magnipa-pillata*	*Hydra vulgaris*	45	XP_002163769	8
pre-mRNA-splicing factor CWC25[b]	29	4	61.0/27.3	10.2/5.5	SRProtein	*Ustilago maydis*	21	EAK85329	5
myb-related protein cdc5[b]	20	4	87.7/27.3	6.4/5.5	SRProtein	*Claviceps purpurea*	17	CCE33636	2
serine/arginine-rich splicing factor 7 isoform 1	18	4	27.4/27.3	11.8/5.5	SRProtein	*M. musculus*	28	NP_666195	7
bud13[b]	16	5	32.5/20.8	9.5/4.8	SRProtein	*Zygosaccharomyces rouxii*	22	CAR30797	5
MUSCLE (n = 2)									
dumpy CG33196-PB	92	8, 10	456/20-20.7	5.3/5.3-5.9	*H. magnipa-pillata*	*H. magnipapillata*	31	XP_002167485	2
dystrophin (isoform 2)	18	5	206/20.8	7.3/4.8	SwissProt	*Drosophila melanogaster*	41	DMDD_DROME	1
Protein quality control (n = 4)									

Table 3. (Continued)

Protein name	Total length of all peptides	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	Score	NCBI accession	Coverage (%)
UDP-glucose: glycoprotein glucosyltransferase 2	131	6, 8	175/20.7-21.1	6.4/5.1-5.3	Selenoprotein	*H. sapiens*	18	NP_064506	9
E3 ubiquitin protein ligase	43	2	59.4/27.3	7.1/5.0	NMMBA	*Meleagris gallopavo*	38	B3RW44	7
ubiquitin carboxyl-terminal hydrolase 17-like protein 10	36	2, 5	59.8/20.8-27.3	8.5/4.8-5.0	Defensin	*H. sapiens*	40	I3L0E4	7
small heat shock protein	35	1	26.4/27.4	5.8/4.9	*H. magnipa-pillata*	*H. magnipapillata*	21	T2MHG6	15
Signaling/hormones (n = 5)									
serine/threonine-protein kinase ATR	46	8	190/20.7	8.3/5.3	*H. magnipa-pillata*	*H. magnipapillata*	27	XP_002160018	3
tyrosine kinase receptor	45	6-9	200/20.4-21.1	5.9/5.1-5.5	*H. magnipa-pillata*	*H. magnipapillata*	33	XP_002169535	3
baculoviral IAP repeat-containing protein	19	1	512/27.4	5.8/4.9	nr other metazoa	*Nasonia vitripennis*	24	K7JAH7	<1
receptor-type tyrosine-protein phosphatase alpha	18	9	90.5/20.4	6.3/5.5	SwissProt	*H. sapiens*	31	78PTPRA_HUMAN	2
serine/threonine-protein kinase PLK4-like	15	9	76.5/20.4	8.9/5.5	nr other metazoa	*S. kowalevskii*	30	XP_002734025	2
transcription (n = 1)									
RNA binding motif protein	69	2-3, 8	55.8/20.7-27.4	10.0/5.0-5.3	TRA2-beta	*H. sapiens*	14	P0DJD3	10
Translation (n = 2)									
ribosomal protein S4	39	6	29.4/21.1	10.3/5.1	*H. magnipa-pillata*	*H. vulgaris*	30	XP_002154886	15
eukaryotic translation initiation factor 3 subunit	17	10	109/20.0	5.3/5.9	SwissProt	*Vanderwaltozyma polyspora*	24	EIF3A_VANPO	2

Protein name	Total length of all peptides	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	Score	NCBI accession	Coverage (%)
Transport (n = 4)									
apolipoprotein	58	3	30.6/27.4	5.6/5.2	SwissProt	*M. musculus*	97	APOA1_MOUSE	21
ATP binding cassette sub-family A	54	4	204/27.3	9.2/5.5	*H. magnipapillata*	*H. magnipapillata*	20	XP_002157466	3
serotransferin	48	3	76.7/27.4	6.9/5.2	SwissProt	*M. musculus*	59	TRFE_MOUSE	7
SEC14-like protein 1	25	2, 10	106/20-27.3	8.5/5.0-5.9	*H. magnipapillata*	*H. magnipapillata*	29	T2M7V3	3
Unknown function (12)									
viral A-type inclusion protein[a]	95	5	332/20.8	5.1/4.8	*H. magnipapillata*	*H. magnipapillata*	61	XP_002156044	3
kinesin motor domain + hypothetical protein	86	1	148/27.4	4.9/4.9	NMMBA	*Bombyx mori*	21	Q237L2	6
retrotransposon-like	47	2	141/27.3	9.2/5.0	*H. magnipapillata*	*H. magnipapillata*	22	T2MJH3	4
uncharacterized protein (putative apolipoprotein)	45	2	477/27.3	8.1/5.0	*H. magnipapillata*	*H. magnipapillata*	24	T2MHF5	1
hypothetical protein	32	5	32.3/20.8	6.0/4.8	Acropora	*A. millepora*	16	ACJ64664	10
selenoprotein	30	8	10.5/20.7	9.7/5.3	Selenoprotein	*H. sapiens*	14	2Q2F_A	33
hypothetical protein	30	3	121/27.4	6.5/5.2	*H. magnipapillata*	*H. magnipapillata*	16	XP_002160564	2
hypothetical protein with galactose binding lectin domain 1	26	4	24.8/27.3	7.5/5.5	Acropora	*A. millepora*	21	ACJ64660	11
hypothetical protein with galactose binding lectin domain 2	20	7	30.9/20.9	6.8/5.3	Acropora	*A. millepora*	14	ACJ64658	6
hypothetical protein	17	3, 5, 8	35.2/20.7-27.4	8.5/4.8-5.3	Acropora	*Acropora tenuis*	14	BAE46797	5
hypothetical protein	16	7	47.9/20.9	5.3/5.3	*H. magnipapillata*	*H. magnipapillata*	26	XP_002163478	3
B-cell CLL/lymphoma 11A	15	3	90.1/27.4	7.6/5.2	nr other metazoa	*Tribolium castaneum*	35	XP_975280	2

[a] May be of bacterial/viral origin.

[b] May be of *Symbiodinium* origin.

De-stained PVDF membranes were blocked in 5% skim milk (w/v) in Tris-buffered saline with Tween-20 (TBST; 100 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20) for 1 hr at RT. The blocking buffer was decanted, and 10 ml of a 1:5000 dilution of a RBCL primary antibody (forms I and II, Agrisera) in 5% skim milk (w/v) in TBST were added to the membranes, which were then incubated for 2 hr with gentle agitation at RT. This antibody has been used successfully for detection of the RBCL of *Symbiodinium* [26], as well as other dinoflagellates [32-33]. Membranes were washed thrice (10 min each) with TBST and then incubated in 10 ml of a 1:5000 dilution of goat anti-rabbit secondary antibody (Millipore, Germany) in TBST for 5 min and washed with TBST as above. Membranes were then stained with 400 µl SuperSignal® West Pico Chemiluminescent Substrate Kit chemiluminescent reagent (Thermo-Scientific), and the chemiluminescent signal was immediately visualized on a Fusion FX7 (Vilber Lourmat, France) gel doc under the chemiluminescence setting.

ImageJ (National Institutes of Health, USA) was used to quantify RBCL protein band intensity, and values (arbitrary units) were first divided by the intensity of the positive control band on the respective gel. These gel-normalized values were then divided by the respective *Symbiodinium* GCP for each sample, which was previously calculated [15] and is routinely used to control for variable ratios in host: *Symbiodinium* biological material between samples [34]. The effects of SO, TT, and the SO x TT interaction on GCP-normalized RBCL expression were tested with a 2-factor ANOVA, which was performed with JMP® (ver. 11.1.1, SAS Institute, USA) after having log-transformed the data due to lack of normality. Then, JMP was used to determine the statistical significance of the correlation between *rbcL* mRNA expression (measured previously [15] in the same samples from which proteins were extracted herein) and the RBCL protein expression quantified herein. It was hypothesized that a significant, positive, linear relationship would be documented between expression levels of these two molecules for both TT (n = 6 protein samples/TT), as well as across the dataset as a whole (n = 12 protein samples), and an α level of 0.05 was set for all aforementioned statistical tests.

RESULTS

Differentially Expressed Proteins Uncovered by a 2D + MS-Based Approach

It is evident from Tables 3 and 4, as well as Figure 3, that a number of different cellular processes were affected by exposure to variable temperature. Of the coral host's differentially expressed proteome (DEP; Table 3), 18 pfam categories could be identified, and these groupings encompassed 62 of the 75 proteins identified (83%). The only functional categories in which more than five proteins were identified with confidence (Figure 3) were DNA binding/transcription factor (n = 8; 10.7%), metabolism (n = 8; 10.7%), and mRNA processing (n = 15; 20%). In contrast, only one transcription factor (2%) was under-expressed in *Symbiodinium* samples of the variable TT (Table 4), and this zinc finger transcription factor was one of only two proteins found in the DEPs of both compartments (the other being the pre-mRNA splicing factor CWC22). However, the degree of homology of these proteins across compartments could not be ascertained due to the short nature of the sequenced peptides (17-20 AA).

Proteins dissolved in a urea + thiourea-based buffer were pooled between the two sites of origin (SO), Houbihu (upwelling site) and Houwan (non-upwelling site), and electrophoresed across two dimensions as described in the text. A representative 2D gel out of the four that were run for each temperature treatment (TT) has been shown for both stable (A) and variable (B) temperature specimens. Although no proteins appeared to have been solely expressed by one treatment, a total of 10 protein spots (circled in C) were found to be over-expressed by samples of the stable TT by image analysis software, and these protein spots were extracted from the gel with sterilized pipet tips (D), processed as described in the text, and submitted for sequencing by mass spectrometry (MS). The y-axis labels in (A) and (C) are shared with (B) and (D), respectively, while the x-axis labels in (C) and (D) are shared with (A) and (B), respectively. "Before" and "after" refer to before and after removing the protein spots, respectively, with sterilized pipet tips. pI = isoelectric point. kDa = kilodalton.

There were only two functional categories that encompassed multiple proteins and were represented in each of the two eukaryotic DEPs of this association: metabolism and mRNA processing. Regarding the latter, although mRNA processing is surely a cellular process that could be hypothesized to undergo differential regulation during periods of temperature change, the fact

that the majority of the proteins aligned most closely to published fungal proteins precluded the ability to confidently ascribe them to one compartment; in fact, many were equally homologous to bacterial proteins (see annotations in Tables 3-4.). Therefore, while these mRNA processing proteins are *likely* involved in the coral and/or *Symbiodinium* response to variable temperatures, their relative importance has been downplayed in this manuscript until longer peptide sequences can be obtained and the compartment of origin more confidently assigned.

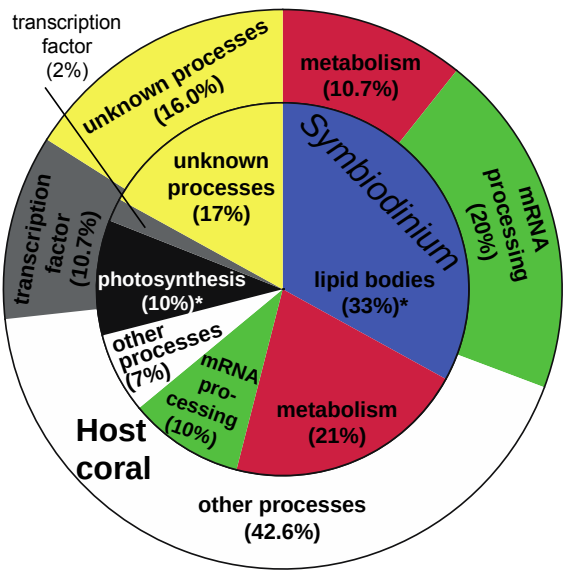

Figure 3. Functional distribution of the host coral and *Symbiodinium* differentially expressed proteins. Percentage breakdown of pfam functional groups encompassing the 75 host coral (outer pie graph) and 42 *Symbiodinium* (inner pie graph) proteins that were over-expressed in samples exposed to a stable temperature regime. Functional categories that were over-represented in the *Symbiodinium* differentially expressed proteome (DEP) relative to the host DEP (2-sample proportion test, $p < 0.05$) have been marked with an asterisk (*).

Regarding the second functional category that featured multiple proteins for both eukaryotic compartments of this reef-building coral, eight and nine metabolism-targeted proteins were over-expressed in stable TT samples of the host coral and *Symbiodinium* compartments, respectively (Figure 3), and one such *Symbiodinium* protein, lipoxygenase, is known to play a role in lipid metabolism. Also pertaining to lipids/lipid metabolism, one process that was

over-represented in the *Symbiodinium* DEP relative to the host coral one was lipid bodies (LBs); nearly 1/3 of the *Symbiodinium* DEP (Figure 3) was comprised of proteins involved in stabilization and metabolism of LBs, notably oleosins and caleosins.

RBCL Western Blot

The *Symbiodinium* populations (clade C only [15, 17]) housed within corals exposed to the variable TT for seven days expressed the RBCL protein (Figure 4) at similar levels between the four SO x TT groups (n = 3; Figure 4B-C), and, furthermore, there was no significant, positive correlation between *rbcL* mRNA and RBCL protein expression across the 12 samples of the SHVTS collected after seven days of treatment exposure (Figure 4D).

CONCLUSION

Curiously, no proteins were uniquely expressed by one treatment and not the other, nor were any proteins expressed at higher levels in samples of the variable TT. One explanation for this could be the low quantity of protein loaded; mini-gels (~10 x 10 cm) were used herein with 100 µg protein, and many spots on the gels were fairly faint. This may have limited the ability to detect proteins that were over-expressed by the variable TT samples, and future studies attempting to look at proteome-scale differences between experimental coral samples may consider loading larger quantities (e.g., 500 µg) of protein into the gels. Furthermore, proteins were pooled across two SO of differing environmental history; although preliminary data revealed that the effect of TT led to greater variation in the *S. hystrix-Symbiodinium* physiological response than did the SO (Table 1; [15-17]), future work should nevertheless seek to determine the extent to which environmental history (i.e., SO) drives the ability of this common, widely distributed coral species to acclimate to future changes in temperature, using samples collected both before and after experimentation. Corals from Houwan, a site that never experiences upwelling *in situ*, were, in contrast to what had been hypothesized, readily able to acclimate to a temperature regime that fluctuated from 23 to 29ºC over a 6-hr period; it would be interesting to know if the protein-level acclimation response differed between these corals and those of Houbihu, which *do* experience such highly variable temperatures *in situ*.

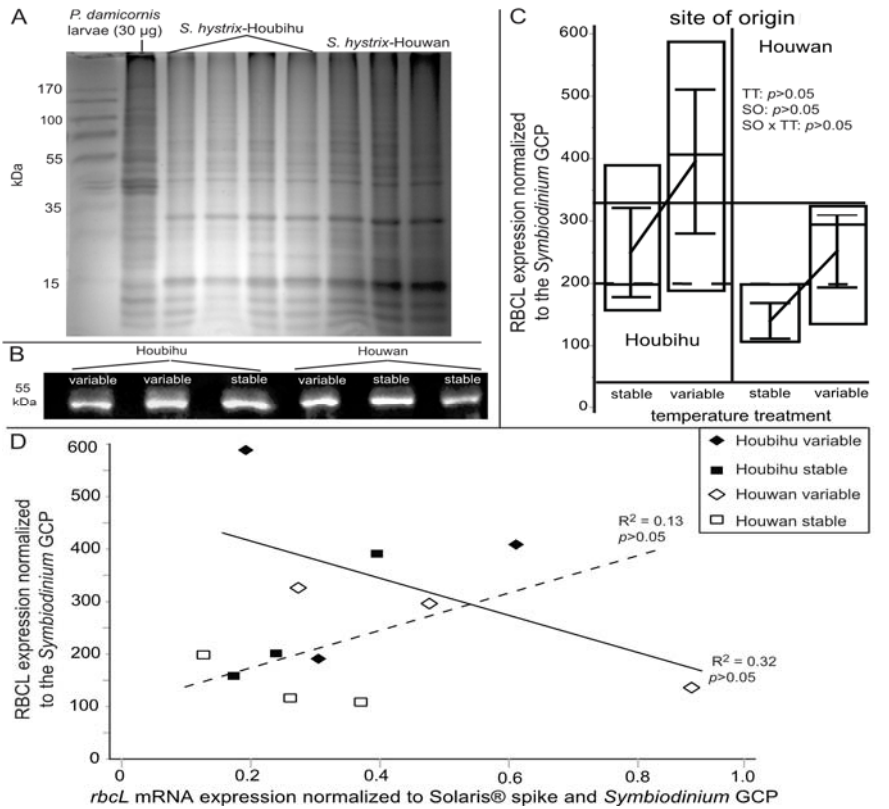

Figure 4. RBCL protein expression. Proteins were electrophoresed as described in the text, and a representative SDS-PAGE gel including a ladder, a positive control sample (30 µg of soluble protein from *Pocillopora damicornis* larvae [7]), and several proteins from each of the two sites of origin (SO) have been shown (A). A 55-kDa protein was detected with an RBCL (forms I and II) antibody, and representative bands from different SO and temperature treatments (TT) have been shown (B). The positive control sample also yielded a ~55-kDa band (data not shown). RBCL expression was normalized to a proxy for *Symbiodinium* density within samples, the genome copy proportion (GCP), and normal quantile plots (with averages connected by solid diagonal lines) for samples of both SO and TT have been shown (C). Error bars represent standard error of the mean (n = 3 for each SO x TT group), and the 2-way ANOVA (SO x TT) *p*-values have been presented on the figure. The global (pooled across SO) variable and stable TT expression levels have been plotted as horizontal solid and dotted lines, respectively. Expression of the RBCL protein (normalized to the *Symbiodinium* GCP) was plotted against expression of the respective *rbcL* mRNA (normalized to both the Solaris® [Thermo-Scientific] RNA spike and the *Symbiodinium* GCP), which was measured in a previous study [15], and best fit lines have been plotted for data of both the variable (solid line) and stable (dotted line) TT (D).

Despite the potentially low resolution of the approach utilized herein, 117 proteins were nevertheless found to be expressed at higher levels in samples exposed to a stable TT for one week relative to those exposed to a variable TT for this same duration. The majority of these proteins (64%) were from the coral host, with the remaining 36% from the *Symbiodinium* populations housed within these samples. This ~2:1 ratio of host/endosymbiont agrees well with biological composition estimates made with other pocilloporids [13]. It should be noted that the fact that the coral host comprises a greater fraction of the holobiont means that differentially expressed proteins will be more readily documented for this compartment due to having loaded a larger quantity of coral protein (i.e., ~64 µg coral host protein/100 µg total holobiont protein) into the gels.

Although *rbcL* mRNA expression was significantly higher in *Symbiodinium* populations harbored within *S. hystrix* colonies of the variable TT [15], the expression of the respective protein was not only similar between TT, but also between SO. As such, it was unsurprising that RBCL was *not* found to be differentially expressed between TT by 2D + MS analysis. Furthermore, *rbcL* gene expression did not correlate positively with RBCL protein expression to a significant degree across the 12 samples. This lack of correlation may suggest that inferring protein expression differences based only on mRNA-scale data, as is common in the coral biology field (e.g., [9-10, 35]), is risky. Additionally, the respective proteins of none of the differentially expressed genes identified in these same samples [15, 17], such as photosystem I (*psI*), were sequenced herein, further pointing to an absence of significant, positive correlation between gene and protein expression in this coral-*Symbiodinium* holobiont. As a final example, expression of no heat shock protein (*hsp*) mRNA was found to be affected by variable temperature exposure in these samples [15, 17], yet a small HSP was found by 2D gel electrophoresis to be down-regulated in host corals exposed to variable temperatures for seven days.

Although next generation mRNA sequencing has yielded marked insight into the molecular biology of cnidarian-dinoflagellate endosymbioses [13], the observation made herein that there is not always a positive association between mRNA and protein expression suggests that researchers should heir on the side of caution when attempting to use their mRNA data to make predictions about how corals will respond to environmental change. Rather than an end-all, such RNA Seq-based endeavors may be better seen as a means to target specific *proteins*, rather than gene mRNAs, for future, molecular characterization studies. Though not without their own limitations, 2D + MS-

based methods yield direct insight into the molecules that actually carry out essential cellular processes; such proteins, of which several are discussed below, may better serve as biomarkers of the coral response to environmental changes, and notably GCC.

Upon a comprehensive look at the proteins that were down-regulated after seven days of exposure to variable temperature in the *S. hystrix-Symbiodinium* holobiont, it appears that different cellular processes were affected in each compartment. From an evolutionary perspective, eukaryotic cells are expected to respond similarly to changes in temperature [14, 36]. However, given the extensive evolutionary divergence between cnidarians and protozoans, it is unsurprising that some unique pathways were differentially affected by variable temperature exposure between the two endosymbiotic constituents. One group that merits further mention is the LB-associated proteins, oleosin and caleosin. The former is an abundant structural protein that acts to stabilize LBs, which are absent from asymbiotic or aposymbiotic cnidarians, by preventing their coalescence [37]. Caleosin is thought to play a role in the degradation of LB lipids in higher plants [38], though its ancestral role appears to be as another structural protein found on/in the coat of LBs [39]. As caleosin and oleosin are plant proteins, they were almost surely synthesized by the *Symbiodinium* cells. However, it cannot be ruled out that these proteins migrate alongside the LBs as they flow between compartments (discussed in more detail below). For the sake of argument, though, the remainder of this discussion will assume that these proteins are ultimately of dinoflagellate origin.

Endosymbiotic anthozoan LBs are thought to be involved in the metabolic dialogue between host anthozoans and their *in hospite Symbiodinium* populations [27, 37]; these organelles have been shown to flow back and forth between hosts and endosymbionts as a means of transferring lipids [24]. It seems reasonable to speculate, then, that the decrease in oleosin and caleosin expression in *Symbiodinium* within corals exposed to variable temperature for seven days may suggest that LB metabolism differed fundamentally between TT; perhaps the down-regulation of these two proteins under variable temperatures insinuates that LBs were being metabolized, since proteins that catabolize LB lipids could more readily interact with LBs upon the absence of these integral coat proteins. This presumed up-regulation of LB metabolism may have allowed for these *Symbiodinium* populations to sustain sufficient energy levels to maintain homeostasis under periods in which cellular energy demand could be hypothesized to be high due to, for instance, elevated rates of protein turnover brought on by these rapid temperature changes.

However, it should be noted that caleosin and oleosin are also known to have roles in lipid metabolism [40-41], and so their down-regulation, alongside a decrease in expression of another lipid-metabolizing protein, lipoxygenase, at variable temperature could, in contrast, suggest a decrease in LB metabolism. Indeed, the overall down-regulation of proteins involved in a variety of cellular pathways, notably metabolism, at variable temperatures in both compartments of this endosymbiosis may ultimately speak to this need to conserve energy for growth, which was similar between temperature regimes (Table 1 and [15]), by suppressing certain metabolic pathways. As evidence for this, *Symbiodinium* have been found to actually *accumulate* lipids and LBs when deprived of nitrogen [42]. Future work should, then, seek to uncover the role of proteins involved in LB stabilization and metabolism in the response of *in hospite Symbiodinium* populations to environmental change; without further immuno-localization studies, it is premature to conjecture where these LB metabolic changes, in fact, took place, within the host coral or within the *Symbiodinium* cells. Such studies should also attempt to observe LB metabolism during stress events in order to determine whether they are more likely to be synthesized/accumulated, as when undergoing nutrient stress, or catabolized at such times. The ensuing data could help to develop a more comprehensive cellular model of how reef-building corals acclimate to highly variable temperature exposure both *in situ* and in the laboratory.

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AUTHOR CONTRIBUTION STATEMENT

A.B.M. conducted the experiment, processed the samples, analyzed the data, and wrote the manuscript. Y.-J.C. and C.-Y.L. ran the 2D gels and sequenced the proteins, respectively. C.-S.C. provided laboratory space, facilities, and reagents that were instrumental to the success of the project. A.B.M. declares that the authors have no competing interests.

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Table S1. Seventy-five host coral proteins expressed at higher levels in specimens of the stable temperature treatment in the 2010 Seriatopora hystrix variable temperature study [15-17]. For proteins found in multiple spots, the range of molecular weights (in kilodaltons [kDa]) and isoelectric points (pI) have been provided

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	Score	NCBI accession	Coverage (%)
CELL SURFACE PROTEINS (n = 4)									
fibrocystin 100	QVTVEVNSIPSTCSKR VTSFSKNNICCLTGAR ILIVDGGR QQPDRVAIAGGR VVQIVNALGDSRR ASDNDPSGGRAR LKESIPELR SMYNFSVIIKFASHK	1	678/27.4	8.2/4.9	NMMBA	*Saccoglossus kowalevskii*	19	A7SPV0	4
integrin α 69	YWAIVGAPLSNGSSGAEKFTR ADVVSGAPR YESGQFSVR NGYADVLVGAPYFTDVLDEGRVYIYLN DGK	7	112/20.9	5.6/5.3	Acropora	*Acropora millepora*	17	ABY7449 8	6
integrin β2 36	EITNITR QAYEKIAK AKPNQCLR DRDTGLLCGGPER	5-6	85.0/ 20.8-21.1	5.3/4.8-5.1	Acropora	*A. millepora*	14	ABY7449 9	4

Table S1. (Continued)

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
H6 G-protein coupled receptor 19	LRAASVEK KIHDTATSSSR	9	34.7/20.4	9.7/5.5	Acropora	*A. millepora*	15	ABI50929	6
CIRCADIAN RHYTHM (n = 1)									
cryptochrome photolyase (CRY2) 91	DLDTSLVECGSR ENGIEVISR QPEKPVPKVGR LDEIGGKFSK GCLSPRLLHQR GVSPPSELFVK SYGCVIGR QRAICVQRMQELAFKLASK	3, 6-7, 10	59.9/20.0-27.4	8.8/5.1-5.9	Acropora	*A. millepora*	16	ABP97099	17
CYTOSKELETON (n = 2)									
ciliary dynein heavy chain 21	SQEAAAIVK ELKNAQELLDSK	5	525/20.8	5.7/4.8	nr other metazoa	*Pediculus humanus corporis*	38	XP_002426312	1
stathmin 19	HHEEQLIAK LEQSAENRK	5	34.7/20.8	9.8/4.8	nr other metazoa	*Trichoplax adhaerens*	50	XP_002114295	5
DEVELOPMENT (n = 2)									
hedgehog 30	KKPIPPTTNITLPR EMEKELK LLLTWNDLS	2	90.9/27.3	6.4/5.0	Acropora	*Acropora cervicornis*		GASU01035919	
embryonic polarity protein dorsal 19	ENMIFENELK SPDSPPNKK	7	93.2/20.9	5.4/5.3	nr other metazoa	*Culex quinquefasci-atus*	42	XP_001844078	2

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
DNA BINDING/TRANSCRIPTION FACTOR (n = 8)									
nuclear receptor AmNR2 63	NRCQYCRFNKCL AQGMLKEAVREDR SLAPRSADAPSSR LGKLLLCLPTLR ALENYVTLEFFGK	3, 8	49.9/ 20.7-27.4	6.0/ 5.2-5.3	Acropora	*A. millepora*	34	AF323681_1	14
sex comb on midleg-like protein 2 50	IQKAELATAR CNQDSRNPDK TTPMKPKTLNQAEVCINIE CVCGPYIDVEK	7-8	117/ 20.7-20.9	8.3/5.3	*Hydra magnipa-pillata*	*Hydra magnipapilla-ta*	34	XP_002160709	3
nuclear receptor 6 49	SSGFHYGVQSCEGCK CLSLGMLKEAVRED RAPGGRPRIK LPTLRHVSSK	1, 2, 10	38.8/ 20-27.4	8.3/ 4.9-5.9	Acropora	*A. millepora*	18	AF323686_1	13
estrogen receptor-β 48	SLEHTLPVNR CASPVTGPGSK SADEQLHCAGKAK IPGFVELSLFDQVR	2, 4	59.2/27.3	8.8/5.0-5.5	Seleno-protein	*H. sapiens*	29	ESR2_HUMAN	8
topoisomerase (DNA) III β 36	GLNPAQLKALYNSGYK MRQEQNK RSNPSEIPADDVK	8	153/20.7	8.6/5.3	*H. magnipa-pillata*	*H. magnipapillata*	23	XP_002155556	2

Table S1. (Continued)

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	Score	NCBI accession	Coverage (%)
zinc finger protein 26	MHTVDANK HTTLDAARGR IATALDPR	10	71.3/20.0	9.2/5.9	*H. magnipa-pillata*	*H. magnipapillata*	32	XP_012561483	4
nuclear receptor AmNR8 18	CQGNGACPVDK EAVQCER	3	42.5/27.4	8.8/5.2	Acropora	*A. millepora*	16	AF323688_1	4
DM domain protein 17	SPPSQSPR SSTTPSIIK	2, 9	51.5/ 20.4-27.3	9.0/5.0-5.5	Acropora	*A. millepora*	16	AF530064_1	3
DNA REPAIR (n = 1)									
Fanconi anemia group J protein 50	QVLTENHRALHVMTGSFLR NTQVMAGGWRR IVSEPRGGDK YTNGISKWVR	2	125/27.3	6.3/5.0	NMMBA	*Danio rerio*	36	K7GJE0	4
GROWTH FACTOR (n = 1)									
imaginal disc growth factor 18	TLLESVESR EIQAGYLGK	3-4, 9	49.4/ 20.4-27.4	6.7/5.2-5.5	nr other metazoa	*Diaprepes abbreviates*	41	AAV68692	4
GLYCOPROTEIN (n = 1)									
mucin-17 98	HSSNKSSGKSSSKSSSGK SSPSKSSSSK QNGSLLKR QHYPVYQLKPK AAAKVLFDINRPSIY ATVENNYIETTSLPK SMLPTQTIDSSKIVTPSSK	9	574/20.4	5.4/5.5	*H. magnipa-pillata*	*H. magnipapillata*	26	XP_002160582	2

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	Score	NCBI accession	Coverage (%)
IMMUNITY (n = 1)									
beta-defensin 22	VTEQLKRCWGEYIR ICRISEIR	2	11.8/27.3	9.1/5.0	Defensin	*Canis lupus*	26	Q30KS7	22
METABOLISM (n = 8)									
thioredoxin reductase (isoform 1) 73	MAVALRGLGGR WRTQAVAGGVR WYDYDLIIIGGGS GGLAAAKEAAQLGRK VPDTRSLNLEK RSGLDPTVTGCUG	3-5	56.2/20.8-27.3	7.2/4.8-5.5	Seleno-protein	*H. sapiens*	15	AF044212_1	14
porphobilinogen deaminase 39	SIRGNLNTR MGWHNRVGQILH PEECMYAVGQGALGVEVR	6	39.3/21.1	6.7/5.1	SwissProt	*H. sapiens*	24	HEM3_HUMAN	11
carboxylesterase 1C 37	AISESGVVINTNVGK APEEILAEK EGASEEETNLSK	3	61.0/27.4	5.0/5.2	SwissProt	*Mus musculus*	55	EST1C_MOUSE	7
carbamoyl-phosphate synthetase/aspartate transcarbamylase/ dihydroorotase 29	KYFSDIVAR LALGIPLPKLK AKQMGFSDK	9	101/20.4	6.8/5.5	*H. magnipa-pillata*	*H. magnipapillata*	19	NP_001267868	3
alpha-L-arabinofuranosidase B 27	HQNFIFK LHSADFNSELYK IVKALNGR	9	35.6/20.4	8.9/5.5	*H. magnipa-pillata*	*H. magnipa-pillata*	28	XP_002165874	8

Table S1. (Continued)

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	*Mascot database*	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
phospholipase D 21	NGEIFGKVFNCIPSSGLLTFR	4	104/27.3	8.5/5.5	nr other metazoa	*Schistosoma mansoni*	38	XP_002576773	2
cytochrome 450 19	LRSGTTLSPFNGAYVGLTR	4	56.9/27.3	9.0/5.5	nr other metazoa	*Trichoplax adhaerens*	38	XP_002112306	3
mannosyl-glycoprotein endo-beta-N-acetylglu- cosamidase[a] 15	EGLTTPEK QWIPTVK	1	37.7/27.4	9.3/4.9	*S. aureus*	*S. aureus*	23	WP_000247512	4
MITOSIS (n = 1) inner centromere protein 17	ESTPIEK LAEKDIAIGK	4	112/27.3	9.3/5.5	*H. magnipa-pillata*	*H. magnipa-pillata*	26	XP_002167890	2
mRNA PROCESSING (n = 15) serine/arginine repetitive matrix protein 2 212	NLSLVRGR QIAPEPPKPYSLVRETSSSR SRSPQRPGWSR GRSGSSSER SNSPQPKVK TPSRQSCSGSSPR SRSISPCPK SISPCPKVDSR HSGSTSPYLK SEISTDPK VGLFSSQK SSSASPELKDGLPR	5	295/20.8	12/4.8	SRProtein	*M. musculus*	22	SRRM2_MOUSE	7

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	*Mascot database*	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
	SRSGSSPGLR ERSGSESSVEQK ALPRHSR TKSHTPPR SPTRQESSR TPLISRR SRSPLAIR SATPPATRNHSGSR TSPLMLDR								
pre-mRNA-splicing factor CWC22 127	LIIQEFLKENIVR FSNIGHLILK MADGTLGR NSLKLIETSR AIKAILGMSK NITEGQIDK TPETFSR FISYLLYYLDTNK FFAHLLFTNSILGKVFCCIK FPFLSIDLVGLTDNLK HLKLYPESAIK	3	65.3/27.4	9.3/5.2	SRProtein	*Bombus impatiens*	36	XP_003494375	22
pre-mRNA splicing factor prp8[b] 67	IVKDIGDVSQK RSYLGALK LLKTYVLNELHK AFNIVSGEDR DVIEATTTNK QLTAVTTK AIATSNLR	4	326/27.3	8.4/5.5	SRProtein	*Tricho-phyton tonsurans*	28	EGE00188	2

Table S1. (Continued)

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
pre-mRNA-splicing factor CDC5/ CEF1[b] 55	EAEQSSKR NIRALTETK KSQIETPNPMATPFR GEVSEEDAEIR DGSAVGAEIFDK	5	87.0/20.8	6.5/4.8	SRProtein	*Pseudogym-noascus destructans*	22	ELR07040	7
transformer-2 protein homolog beta-like 52	LQPLLSDHRR HYVLAPR GVHLSVSR SPTPRSR QDDADAAR LNGSELDGRSIR	7	82.2/20.9	11.6/5.3	TRA2-beta	*Salpingoeca rosetta*	14	EGD83325	7
pre-mRNA-splicing ATP-dependent RNA helicase prp28[b] 51	NSEVPTGPAAMRNK QKYMGTEK AREILEMER AAIPIALQSR SDDSSGFGNK	5	88.3/20.8	8.8/4.8	SRProtein	*Neosartorya fischeri*	35	PRP28_NEOFI	6
ATP-dependent RNA helicase DHX8-like 51	LEPEVTHVKDK FGCFVQLEGLRK QSLDLSPVR TLVDGQVVYIHPSSALFNR	3	137/27.4	8.5/5.2	SRProtein	*S. kowalevskii*	22	XP_002734274	4
CWC25 spliceosome-associated protein homolog 42	ELEEERAR DLLNNPVKMK SEWHKDSR ESTSPQRSR	5	58.1/20.8	10.0/4.8	SRProtein	*S. kowalevskii*	17	XP_002732008	8

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
	QTRSHSR								
pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16 37	SKISSYK DWEEGKSDSGS DEEDDEENK YGMVGCTQPR	5	144/20.8	6.4/4.8	SRProtein	*Oryzias latipes*	27	XP_004067034	3
pre-mRNA-splicing factor SYF1[b] 32	SVQAQYNTEVSFL AAQAQGAK VALEKELATAK	4	41.8/27.3	5.5/5.5	SRProtein	*Rhizoctonia solani*	31	CCO31421	8
KH domain containing, RNA-binding, signal transduction-associated 3 29	VVVPVKEYPK GRGFASAPIVR HASTAPDR	5	38.3/20.8	6.5/4.8	*H. magnipa-pilla*	*Hydra vulgaris*	45	XP_002163769	8
pre-mRNA-splicing factor CWC25[b] 29	IREDPMLAIK LHDADDSRPASR QQARQLK	4	61.0/27.3	10.2/5.5	SRProtein	*Ustilago maydis*	21	EAK85329	5
myb-related protein cdc5[b] 20	GGVWTNIEDEILK MHEVALR	4	87.7/27.3	6.4/5.5	SRProtein	*Claviceps purpurea*	17	CCE33636	2
serine/arginine-rich splicing factor 7 isoform 1 18	SISRPRSSR SRSPSGSPHR	4	27.4/27.3	11.8/5.5	SRProtein	*M. musculus*	28	NP_666195	7
bud13[b] 16	SQETVFR EPVSLMGRK	5	32.5/20.8	9.5/4.8	SRProtein	*Zygosacchar-omyces rouxii*	22	CAR30797	5

Table S1. (Continued)

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
MUSCLE (n = 2)									
dumpy CG33196-PB 92	DCPSGSYCPNR QTSCIICEAGR CALCSLGYRSNSNK GYYSHEK TYSNTEGATNNQDCR ISDCSPCPGGR ETGYGGVCPIGSFCK YGAKTASPR	8, 10	456/ 20-20.7	5.3/5.3-5.9	*H. magni papillata*	*H. magnipa-pillata*	31	XP_012556696	2
dystrophin (isoform 2) 18	NGFQILDDR ELTEWVIRK	5	206/20.8	7.3/4.8	SwissProt	*Drosophila melanogaster*	41	DMDD_DROME	1
PROTEIN QUALITY CONTROL (n = 4)									
UDP-glucose: glycoprotein glucosyltransferase 2 131	MAPAKATNVVR IAVNQHMR LFINGLR KAGASFYK MMDASVYLQREVFLGTLNDR INTLILR KLLFNALK EEIATAIYSGDK VDALMSSVPKR IINMKIK EDILTDEDEK	6, 8	175/ 20.7-21.1	6.4/5.1-5.3	Seleno- protein	*H. sapiens*	18	NP_064506	9

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
	FWLLKNYLSPTFK								
E3 ubiquitin protein ligase 43	VCSTVEQYFKVIINALNNR IINDINHLGNIKEK IYVADSGNSR	2	59.4/27.3	7.1/5.0	NMMBA	*Meleagris gallopavo*	38	B3RW44	7
ubiquitin carboxyl-terminal hydrolase 17-like protein 10 36	ACLPGHKQVDR HSESVSRGR ALGVEDTDR FLQEQNK	2, 5	59.8/ 20.8-27.3	8.5/4.8-5.0	Defensin	*H. sapiens*	40	I3L0E4	7
small heat shock protein 35	YYPLFNVGTGALAK VEGQTLEVSGKHR SQVQAPLK	1	26.4/27.4	5.8/4.9	*H. magni-papillata*	*H. magni-papillata*	21	T2MHG6	15
SIGNALING/HORMONES (n = 5)									
serine/threonine-protein kinase ATR 46	IALKSIICLFNIMGAK CLGILGAIDPGR LIYSLGKTSCK EPILNMR	8	190/20.7	8.3/5.3	*H. magni-papillata*	*H. magni-papillata*	27	XP_002160018	3
tyrosine kinase receptor 45	RYSEVALR LSSSVFSKK EINLMKEIPYHK ERVDALER YTNPKYSK	6-9	200/ 20.4-21.1	5.9/5.1-5.5	*H. magni papillata*	*H. magni papillata*	33	XP_012562267	3

Table S1. (Continued)

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	*Mascot database*	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
tyrosine kinase receptor 45	RYSEVALR LSSSVFSKK EINLMKEIPYHK ERVDALER YTNPKYSK	6-9	200/ 20.4-21.1	5.9/5.1-5.5	*H. magni papillata*	*H. magni papillata*	33	XP_012562267	3
baculoviral IAP repeat-containing protein 19	ALQFDTYEMIVENPDGGFK	1	512/27.4	5.8/4.9	nr other metazoa	*Nasonia vitripennis*	24	K7JAH7	<1
receptor-type tyrosine-protein phosphatase alpha 18	QAGSHSNSKQAGSHSNSFR	9	90.5/20.4	6.3/5.5	SwissProt	*H. sapiens*	31	78PTPRA_HUMAN	2
serine/threonine-protein kinase PLK4-like 15	NAVVSISCLNLTQYR	9	76.5/20.4	8.9/5.5	nr other metazoa	*S. kowalevskii*	30	XP_002734025	2
TRANSCRIPTION (n = 1)									
RNA binding motif protein 69	NRSPSGSLR MSYSRGLIPVK ATISSWR DEHSSRGYRNHR RHESYSR DYAPPHR NPPSLGR NRSPSGSLR	2-3, 8	55.8/20.7-27.4	10.0/5.0-5.3	TRA2-beta	*H. sapiens*	14	P0DJD3	10

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	*Mascot database*	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
TRANSLATION (n = 2)									
ribosomal protein S4 39	LNAPKHWMLDK VGIITNRER DVTGNQFATRLSNIFLIGK	6	29.4/21.1	10.3/5.1	*H. magnipa-pillata*	*H. vulgaris*	30	XP_002154886	15
eukaryotic translation initiation factor 3 subunit 17	AQGPSASTEAPDDEGR	10	109/20.0	5.3/5.9	SwissProt	*Vanderwal-tozyma polyspora*	24	EIF3A_VANPO	2
TRANSPORT (n = 4)									
apolipoprotein 58	VAPLGAELQESAR LQELQGR LSPVAEEFR THVDSLR TQLAPHSEQMR SNPTLNEYHTR	3	30.6/27.4	5.6/5.2	SwissProt	*M. musculus*	97	APOA1_MOUSE	21
ATP binding cassette sub-family A 54	QGSEVAELPLLQNH AQSVICSPPRSK YGALGFGDLQSTITK VLSGGADNDLLR	4	204/27.3	9.2/5.5	*H. magni-papillata*	*H. magni-papillata*	20	XP_002157466	3
serotransferin 48	GTDFQLNQLEGK DGGGDVAFVK IPSHAVVAR VAQEHFGK LPEGTTPEK	3	76.7/27.4	6.9/5.2	SwissProt	*M. musculus*	59	TRFE_MOUSE	7

Table S1. (Continued)

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	*Mascot database*	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
SEC14-like protein 1 25	SIISSLQLHK NPVNIEK QTVKAQNK	2, 10	106/ 20-27.3	8.5/5.0-5.9	*H. magni-papillata*	*H. magni-papillata*	29	T2M7V3	3
UNKNOWN FUNCTION (n = 13)									
viral A-type inclusion protein[a] 95	ETQKLTIIEK EIHQFQKMGHTK EIESQIKALK QTLVSKLENIEK IVEITVEIKK ESELLINLELEKK DSLEDYLR ALLIKTQNEER IPSPRIDIR	5	332/20.8	5.1/4.8	*H. magni-papillata*	*H. magni-papillata*	61	XP_002156044	3
kinesin motor domain + hypothetical protein 86	TSLVVCVSPTMSDVS ETKSSLYFGSR IIAKLEMDK KAMDVLEEPK LKAELTMSNIQTK ESVHEATRDRRAAA SLLVSTRQERAELNR	1	148/27.4	4.9/4.9	NMMBA	*Bombyx mori*	21	Q237L2	6
retrotransposon-like 47	ALEMIQSGASPK ARHFLLGR TPPYHPQSNGAAERMVETVK SDMSELR	2	141/27.3	9.2/5.0	*H. magni-papillata*	*H. magni-papillata*	22	T2MJH3	4

Protein name Total length of all peptides mapping to one protein	Sequence(s)	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	*Mascot database*	*Taxon (top Mascot hit)*	Score	NCBI accession	Coverage (%)
uncharacterized protein (putative apolipoprotein) 45	NCEGQENYERVEEILMSLK SINLNAKTVSR SNILSTAEELSKALK	2	477/27.3	8.1/5.0	*H. magni-papillata*	*H. magni-papillata*	24	T2MHF5	1
hypothetical protein 32	AAQQFIAKKPK EDESSLTTRVSK FTKEPPLK	5	32.3/20.8	6.0/4.8	Acropora	*A. millepora*	16	ACJ64664	10
selenoprotein 30	IHHHHHHSSGR QEALAAAR KQEELNAQVEK	8	10.5/20.7	9.7/5.3	Seleno-protein	*H. sapiens*	14	2Q2F_A	33
hypothetical protein 30	NIDKANMVAK NSVSEMNPIREAK EATEYAK	3	121/27.4	6.5/5.2	*H. magni-papillata*	*H. magni-papillata*	16	XP_002160564	2
hypothetical protein with galactose binding lectin domain 1 26	KADTGLPR VIKINNAFWGR AALQRSR	4	24.8/27.3	7.5/5.5	Acropora	*A. millepora*	21	ACJ64660	11
hypothetical protein with galactose binding lectin domain 2 20	DDHVTCPK LCETSADNTIDK	7	30.9/20.9	6.8/5.3	Acropora	*A. millepora*	14	ACJ64658	6
hypothetical protein 17	MPAITLR NNVEHQQDPK	3, 5, 8	35.2/20.7-27.	8.5/4.8-5.3	Acropora	*Acropora tenuis*	14	BAE46797	5
hypothetical protein 16	EIDIVIPK EKFGLQAK	7	47.9/20.9	5.3/5.3	*H. magni-papillata*	*H. magni-papillata*	26	XP_002163478	3
B-cell CLL/lymphoma 11A 15	DNNNSTSLTNQLKLR	3	90.1/27.4	7.6/5.2	nr other metazoa	*Tribolium castaneum castaneum*	35	XP_975280	2

[a] May be of bacterial/viral origin.

[b] May be of *Symbiodinium* origin.

Table 4. Forty-two *Symbiodinium* proteins expressed at higher levels in specimens of the stable temperature treatment in the 2010 *Seriatopora hystrix* variable temperature study. For proteins found in multiple spots, the range of molecular weights (in kilodaltons [kDa]) and isoelectric points (pI) have been provided. The average total length of all peptides mapping to a unique protein was 36 ± 21 (standard deviation) amino acids (AA), statistically similar to that of the host coral (43 ± 33; student's *t*-test, $p > 0.05$), and the total number of mapped AA per protein ranged from 16 to 135 AA (polyADP ribose polymerase). Coverage averaged 10.2 ± 6.4%, statistically similar to that of the coral host (7.4 ± 8.1; $p > 0.05$), and ranged from 2 to 25%. Please see Table S2 for the associated peptide sequences

Protein name	Total length of all peptides	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	score	NCBI accession	Coverage (%)
DNA Binding/transcription Factor (n = 1)									
zinc finger transcription factor PEI1	20	1	28.2/27.4	6.8/4.9	32010	*Boechera stricta*	16	Q6WEQ1	8
DNA replication/repair (n = 2)									
polyADP ribose polymerase	135	1	287/27.4	5.9/4.9	SRProtein	*Acanthamoeba castellanii*	45	L8HDR5	5
chromosome segregation protein[a]	20	10	9.4/20.0	4.2/5.9	*Staphylococcus aureus*	*S. aureus*	21	WP_000294226	24
Extracellular matrix protein (n = 1)									
tenascin precursor	18	1	58.5/27.4	9.8/4.9	NMMBA	*Hordeum vulgare*	25	A7RGF8	3
Lipid bodies (n = 14)									
caleosin-related[b]	70	5-7	36.5/20.8-21.1	5.2/4.8-5.3	32011	*Moniliophthora perniciosa*	14	EEB94283	19
Caleosin	53	2, 5	23.8/20.8-27.3	9.6/4.8-5.0	32011	*Arabidopsis thaliana*	16	NP_173738	25
caleosin-related	51	5, 8	30.1/20.7-20.8	8.7/4.8-5.3	32011	*Setaria italic*	29	XP_004985161	18
caleosin-related[b]	49	5	60.3/20.8	8.9/4.8	32011	*Mucor circinelloides*	16	EPB88832	8
Oleosin	33	2, 7, 9-10	17.5/20.0-27.3	9.7/5.0-5.9	32010	*Persea americana*	19	AGT63296	21
caleosin-related[b]	28	7, 9-10	25.5/20.0-20.9	7.2/5.3-5.9	32011	*Parastagonospor nodorum*	20	EAT77728	12

Protein name	Total length of all peptides	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	score	NCBI accession	Coverage (%)
caleosin-related[b]	24	8	28.5/20.7	7.1/5.3	32011	*Sporisorium reilianum*	16	CBQ73148	9
oleosin	23	10	17.5/20.0	9.7/5.9	32010	*P. americana*	16	AGT63296	15
18 kDa oleosin	22	9	28.2/20.4	11.2/5.5	32010	*Zea mays*	13	ACG48647	8
Oleosin	22	1	23.6/27.4	11.3/4.9	32010	*Brassica oleracea*	16	AAD24547	10
oleosin S4-4	20	6	23.0/21.1	9.1/5.1	32010	*Brassica napus*	14	ACG69510	9
pollen coat oleosin-glycine-rich protein	19	1, 5, 7-8	21.8/ 20.7-27.4	9.7/4.8-5.3	32010	*Sisymbrium irio*	25	Q6V5I9	8
steroleosin-B	17	10	39.1/20.0	6.2/5.9	32012	*Oryza sativa*	13	AAT77030	5
18.2 kDa oleosin	16	5	17.9/20.8	9.3/4.8a	32010	*Gossypium hirsutum*	18	AAA18524	9
Metabolism (n = 9)									
peptide methionine sulfoxide reductase	54	2, 6, 8	28.4/20.7-27.3	8.9/5.0-5.3	32010	*B. napus*	19	P54151	21
fructose-bisphosphate aldolase, chloroplastic-like[a]	42	10	33.0/20.0	4.9/5.9	*S. aureus*	*S. aureus*	19	YP_005326917	14
threonine synthase[a]	42	5, 8, 9	37.8/ 20.4-20.8	6.0/4.8-5.5	*S. aureus*	*S. aureus*	28	WP_001581605	12
deoxyribose-phosphate aldolase 1[a]	33	5	23.5/20.8	4.7/4.8	*S. aureus*	*S. aureus*	26	WP_001617202	15
succinyl-diaminopimelate desuccinylase[a]	32	6	45.1/21.1	4.6/5.1	*S. aureus*	*S. aureus*	16	YP_041474	8
acetyl-CoA acetyltransferase[a]	31	5	41/20.8	6.2/4.8	*S. aureus*	*S. aureus*	23	WP_001070664	8
8-hydroxyquercetin 8-O-methyltransferase-like isoform 1	23	2, 6	40.6/ 21.1-27.3	5.3/5.0-5.1	nr plant	*Glycine max*	39	XP_003527405	6
hydroxyethylthiazole kinase[a]	22	8	28.4/20.7	4.6/5.3	*S. aureus*	*S. aureus*	28	WP_001108492	8
lipoxygenase	21	1, 6	95.3/21.1-27.4	7.2/4.9-5.1	nr plant	*O. sativa*	34	NP_001055143	2

Table 4. (Continued)

Protein name	Total length of all peptides	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon (top Mascot hit)	score	NCBI accession	Coverage (%)
mRNA processing (n = 4)									
pre-mRNA-splicing factor ATP-dependent RNA helicase PRP43	63	5	81.2/ 20.8	9.6/4.8	SRProtein	*Chondrus crispus*	37	CDF37591	9
mRNA processing-related protein	41	1, 3-5	65.3/ 20.8-27.4	5.7/4.8-5.5	SRProtein	*Cryptococcus neoformans*	19	P0CR52	7
putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX16[a]	28	3	119/ 27.4	5.8/5.2	SRProtein	*Galdieria sulphuraria*	27	EME29213	2
pre-mRNA-splicing factor CWC22[a,b]	18	4	64.9/ 27.3	5.1/5.5	SRProtein	*Entamoeba dispar*	36	EDR21607	3
Photosynthesis (n = 4)									
light harvesting protein, isoform 1	54	4, 7	29.0/ 20.9-27.3	8.0/5.3-5.5	Acropora	*Symbiodinium sp.*	15	CBI83412	18
light harvesting protein, isoform 2	46	6	49.3/ 21.1	8.9/5.1	Acropora	*Symbiodinium sp.*	45	CBI83416	9
light harvesting protein, isoform 3	41	7-8	26.7/ 20.7-20.9	9.1/5.3	Acropora	*Symbiodinium sp.*	58	CBI83417	12
light harvesting protein, isoform 4	39	9	44.8/ 20.4	8.7/5.5	Acropora	*Symbiodinium sp.*	28	CBI83414	9
Unknown function (n = 7)									
predicted protein	54	2-3	121/ 27.3-27.4	8.0/5.0-5.2	NMMBA	*Triticum urartu*	38	AOTI010780946	4
hypothetical protein	31	4, 10	171/ 20-27.3	6.1/5.5-5.9	nr plant	*Physcomitrella patens*	42	XP_001783424	2

Protein name	Total length of all peptides	Spot(s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	*Taxon (top Mascot hit)*	score	NCBI accession	Coverage (%)
leucine-rich repeat-containing protein	30	2	138/ 27.3	6.8/5.0	NMMBA	*Capsaspora owczarzaki*	33	A0A086TKY0	5
hypothetical protein	30	10	61.7/ 20.0	9.4/5.9	nr plant	*Medicago truncatula*	45	XP_003603005	6
glycine-rich peptide	30	8	11.3/ 20.7	9.2/5.3	nr plant	*M. trunculata*	41	XP_003616789	23
agglutinin	28	2, 6	34.9/ 21.1-27.3	6.8/5.0-5.1	BSA	*Amaranthus caudatus*	16	1JLY_A	9
hypothetical protein	16	6	68.6/ 21.1	6.9/5.1	nr plant	*Populus balsamifera*	39	XP_002298703	3

[a] May be of bacterial/viral origin.
[b] May be of host coral origin.

Table S2. Forty-two *Symbiodinium* proteins expressed at higher levels in specimens of the stable temperature treatment in the 2010 *Seriatopora hystrix* variable temperature study. For proteins found in multiple spots, the range of molecular weights (in kilodaltons [kDa]) and isoelectric points (pI) have been provided. The longest individual peptide lengths for the *Symbiodinium* and host coral compartments were 56 amino acids (AA; pre-mRNA-splicing factor ATP-dependent RNA helicase PRP43) and 31 AA (porphobilinogen deaminase), respectively

Protein name total number of AAs	Sequence(s)	Spot (s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon	score	NCBI accession	Coverage (%)
DNA BINDING/TRANSCRIPTION FACTOR (n = 1)									
zinc finger transcription factor PEI1 20	GGNGDGVAMRLDGEDYDTSR	1	28.2/27.4	6.8/4.9	32010	*Boechera stricta*	16	Q6WEQ1	8
DNA REPLICATION/REPAIR (n = 2)									
polyADP ribose polymerase 135	NALKSTIVAHGGK VVAAECRIGSDR ENTAANALPAK LSLAQLEK LELVQLMK HTIESTR NIFEVCR HGGSDVEVPLK NDSEHAIEAK HTLRTLAR GVPGARGGGFR GGFGGRGGSGGFR GGASLALGEDGFRGGR	1	287/27.4	5.9/4.9	SRProtein	*Acanth-amoeba castellanii*	45	L8HDR5	5
chromosome segregation protein[a] 20	AQAFDEILEGMTNAIQHPVK	10	9.4/20.0	4.2/5.9	*S. aureus*	*S. aureus*	21	WP_000294226	24

Protein name total number of AAs	Sequence(s)	Spot (s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon	score	NCBI accession	Coverage (%)
EXTRACELLULAR MATRIX PROTEIN (n = 1)									
tenascin precursor 18	LAHHSSR CDNRGISKPDR	1	58.5/27.4	9.8/4.9	NMMBA	*Hordeum vulgare*	25	A7RGF8	3
LIPID BODIES (n = 14)									
caleosin-related[b] 70	MNTLSSGATSL GKGVGDGHPLDK GAGDAGQAAFNGA GNAASGAGDVGK EALQVAGKPPTFFD PDADGVVK	5-7	36.5/ 20.8-21.1	5.2/4.8- 5.3	32011	*Monilio-phthora perniciosa*	14	EEB94283	19
caleosin 53	MSHQTVALASKAK GFSPLFPIDVKNSHL CMHGSDTDVYDDDGR NGLLSEKSVR	2, 5	23.8/ 20.8-27.3	9.6/4.8- 5.0	32011	*Arabidopsis thaliana*	16	NP_173738	25
caleosin-related 51	TLQLVSSLPAR LAVPHLRR DSRGLSVLQQHAAFFDR FDAIFSK DKDGLLQR	5, 8	30.1/ 20.7-20.8	8.7/4.8- 5.3	32011	*Setaria italic*	29	XP_004985161	18
caleosin-related[b] 49	QPTSSFLKSHEESSIQK FEALFSKYAKSDTSAK DHALPRALR EHDDPSK	5	60.3/20.8	8.9/4.8	32011	*Mucor circinell-oides*	16	EPB88832	8
oleosin	MADQPKTIK	2, 7, 9-10	17.5/	9.7/5.0- 5.9	32010	*Persea americana*	19	AGT63296	21

Table S2. (Continued)

Protein name total number of AAs	Sequence(s)	Spot (s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon	score	NCBI accession	Coverage (%)
33	DMAQQITKRVQN MGNSEILFSENR		20.0-27.3						
caleosin-related[b] 28	DGIISPYDTFIGFHR HGGDTGAYDNEGR	7, 9-10	25.5/ 20.0-20.9	7.2/5.3-5.9	32011	*Parasta-gonospor nodorum*	20	EAT77728	12
caleosin-related[b] 24	LVNGASKGEVAR CKHGSDSESYDR	8	28.5/20.7	7.1/5.3	32011	*Sporiso-rium reilianum*	16	CBQ73148	9
oleosin 23	DMAQQITK VQNMGNSEILFSENR	10	17.5/20.0	9.7/5.9	32010	*P. americana*	16	AGT63296	15
18 kDa oleosin 22	GARGPQDRAGRNGH PGQGAGGR	9	28.2/20.4	11.2/5.5	32010	*Zea mays*	13	ACG48647	8
oleosin 22	IIINRIK HLHRPNK IYDSETKK	1	23.6/27.4	11.3/4.9	32010	*Brassica oleracea*	16	AAD24547	10
oleosin S4-4 20	GSVPDQLEYAK GLETRTAAA	6	23.0/21.1	9.1/5.1	32010	*Brassica napus*	14	ACG69510	9
pollen coat oleosin-glycine-rich protein 19	FNIFLNLFSFLFPLLDVLK	1, 5, 7-8	21.8/ 20.7-27.4	9.7/4.8-5.3	32010	*Sisymbrium irio*	25	Q6V5I9	8

Protein name total number of AAs	Sequence(s)	Spot (s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon	score	NCBI accession	Coverage (%)
steroleosin-B 17	NANLVLVAR AAQQHKLE	10	39.1/20.0	6.2/5.9	32012	*Oryza sativa*	13	AAT77030	5
18.2 kDa oleosin 16	MAEVRDR EVGQKIENK	5	17.9/20.8	9.3/4.8a	32010	*Gossypium hirsutum*	18	AAA18524	9
METABOLISM (n = 9)									
peptide methionine sulfoxide reductase 54	AALSLSKRAKPTSPFPKTAR SPMNNLFTR QGNDVGTRYR SGIYFYTDEQEKLAR	2, 6, 8	28.4/ 20.7-27.3	8.9/5.0-5.3	32010	*B. napus*	19	P54151	21
fructose-bisphosphate aldolase, chloroplastic-like[a] 42	LTIPTEPNLYKDLAEHPNVVR EEFDKALGDAVESIYDASVNK	10	33.0/20.0	4.9/5.9	*S. aureus*	*S. aureus*	19	YP_005326917	14
threonine synthase[a] 42	IAICASTGNTSASAAAYAARAGLK VVAILTGNGLK DSIIDYIK	5, 8, 9	37.8/ 20.4-20.8	6.0/4.8-5.5	*S. aureus*	*S. aureus*	28	WP_001581605	12
deoxyribose-phosphate aldolase 1[a] 33	ASELTKAAGADFVK IGTSAGVQIMQGLEADSDY	5	23.5/20.8	4.7/4.8	*S. aureus*	*S. aureus*	26	WP_001617202	15
acetyl-CoA acetyltransferase[a] 31	MNQAVIVAAK ETMIASMGIGGGLGNAALFIRF	5	41/20.8	6.2/4.8	*S. aureus*	*S. aureus*	23	WP_001070664	8
succinyl-diaminopimelate desuccinylase[a] 32	SEILKVNEHR DDIFVSALVGATDASSFLGDNK	6	45.1/21.1	4.6/5.1	*S. aureus*	*S. aureus*	16	YP_041474	8
8-hydroxyquercetin 8-O-methyltransferase-like isoform 1 23	CKGVFSGLESLVDVGGGTGTMAK	2, 6	40.6/ 21.1-27.3	5.3/5.0-5.1	nr plant	*Glycine max*	39	XP_003527405	6
hydroxyethylthiazole kinase[a] 22	AIVLANGSPLLAR IQEVEKYV	8	28.4/20.7	4.6/5.3	*S. aureus*	*S. aureus*	28	WP_001108492	8

Table S2. (Continued)

Protein name total number of AAs	Sequence(s)	Spot (s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon	score	NCBI accession	Coverage (%)
lipoxygenase 21	ADLYGKPPQPAADARVMDELK	1, 6	95.3/ 21.1-27.4	7.2/4.9-5.1	nr plant	*O. sativa*	34	NP_001055143	2
mRNA PROCESSING (n = 4)									
pre-mRNA-splicing factor ATP-dependent RNA helicase PRP43 63	SLPVRQK LTNAGVLMS RLPVPPMLARCLLESLR VGCVESMIGV AAVLSVEGAILMSPTAKR	5	81.2/20.8	9.6/4.8	SRProtein	*Chondrus crispus*	37	CDF37591	9
mRNA processing-related protein 41	LANAASSASALLASSNER DRWNGYDPASYK ERESLAEQHLK	1, 3-5	65.3/ 20.8-27.4	5.7/4.8-5.5	SRProtein	*Cryptococcus neoformans*	19	P0CR52	7
putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX16[a] 28	LMERDSK ELSRQEYLK SLHTDILMALVK	3	119/27.4	5.8/5.2	SRProtein	*Galdieria sulphuraria*	27	EME29213	2
pre-mRNA-splicing factor CWC22[a,b] 18	QSFMALK GKGLFVNSVIR	4	64.9/27.3	5.1/5.5	SRProtein	*Entamoeba dispar*	36	EDR21607	3
PHOTOSYNTHESIS (n = 4)									
light harvesting protein, isoform 1 54	RAEPVSAAAAAAAAAKAAAAAK KGDEAGFRNLRAAEIK AAMMAALGAVVQHYVK	4, 7	29.0/ 20.9-27.3	8.0/5.3-5.5	Acropora	*Symbiodinium sp.*	15	CBI83412	18

Protein name total number of AAs	Sequence(s)	Spot (s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	*Taxon*	score	NCBI accession	Coverage (%)
light harvesting protein, isoform 2 46	ASTASSSSGNPSR FADVPNGLAAISK VLTASDPAEK	6	49.3/21.1	8.9/5.1	Acropora	*Symbiodinium sp.*	45	CBI83416	9
light harvesting protein, isoform 3 41	KFSAMASK FADVPNGLAAISK VLTASDPAEK KLNAEIANGR	7-8	26.7/ 20.7-20.9	9.1/5.3	Acropora	*Symbiodinium sp.*	58	CBI83417	12
light harvesting protein, isoform 4 39	TASLAVAGVAMAALAAGGR VLTASDPAEK KLSAELANGR	9	44.8/20.4	8.7/5.5	Acropora	*Symbiodinium sp.*	28	CBI83414	9
UNKNOWN FUNCTION (n = 7)									
predicted protein 54	QSTSKLMATGR LKLDAQCK TAVGNTHSK MLSSYALDNSV FPSEDGTSVESLTLK	2-3	121/ 27.3-27.4	8.0/5.0-5.2	NMMBA	*Triticum urartu*	38	AOTI010780946	4
hypothetical protein 31	AFFNQIVVAPRAGLILVANAK SISPAKVTDK	4, 10	171/ 20-27.3	6.1/5.5-5.9	nr plant	*Physcomitrella patens*	42	XP_001783424	2
leucine-rich repeat-containing protein 30	NGLSVSDK GANSLSQALR VNTSLTSLDLSR	2	138/27.3	6.8/5.0	NMMBA	*Capsaspora owczarzaki*	33	EFW43177	5
hypothetical protein 30	LALQQDVDR MKLEHLEAASVNNANPNDSTK	10	61.7/20.0	9.4/5.9	nr plant	*Medicago truncatula*	45	XP_003603005	6

Table S2. (Continued)

Protein name total number of AAs	Sequence(s)	Spot (s)	Predicted/ actual mass (kDa)	Predicted/ actual pI	Mascot database	Taxon	score	NCBI accession	Coverage (%)
glycine-rich peptide 30	GGGGFGGGSGGGEGGG GGRGGGGYGGGGGR	8	11.3/20.7	9.2/5.3	nr plant	*M. trunculata*	41	XP_003616789	23
agglutinin 28	TAGLPVIMCLKSNNHQK DVFHVIDWK	2, 6	34.9/ 21.1-27.3	6.8/5.0-5.1	BSA	*Amaranthus caudatus*	16	1JLY_A	9
hypothetical protein 16	KEGSMSLADVCDFGHR	6	68.6/21.1	6.9/5.1	nr plant	*Populus balsamifera*	39	XP_002298703	3

[a] May be of bacterial/viral origin.

[b] May be of host coral origin.