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REPORT

Physiological diversity among sympatric, conspecific endosymbionts of coral (*Cladocopium* C1^{acro}) from the Great Barrier Reef

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Abstract Most of the scleractinian corals living in the photic zone form an obligate symbiosis with dinoflagellates in the family Symbiodiniaceae that promotes reef accretion and niche diversification. However, sea surface temperature surpassing the normal summer average disrupts the symbioses, resulting in coral bleaching and mortality. Under climate warming, temperature anomalies and associated coral bleaching events will increase in frequency and severity. Therefore, it is imperative to better understand the variability in key phenotypic traits of the coral-Symbiodiniaceae association under such high temperature stress. Here, we describe the extent of genetically fixed differences in the *in vitro* acclimatory response of four conspecific strains of the common coral endosymbiont, *Cladocopium* C1^{acro}. (formerly *Symbiodinium* type C1); these strains were isolated from *Acropora* corals from inshore sites on the Great Barrier Reef. We characterised algal growth and thylakoid membrane stability under different thermal scenarios and demonstrate previously undocumented physiological diversity among strains of a single Symbiodiniaceae species. Our results have important implications in terms of the perceived accuracy by which environmental stress tolerance of the coral holobiont can be predicted, potentially explaining patchiness in a coral community during bleaching based on the dominant Symbiodiniaceae genotype harboured by the host.

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Introduction

Dinoflagellates in the family Symbiodiniaceae (formerly the genus *Symbiodinium*) comprise a highly diverse group of unicellular eukaryotic algae, most of which form an endosymbiotic association with a wide range of marine invertebrates, including scleractinian corals (Trench 1997; LaJeunesse et al. 2018). Recent studies have estimated that the timing of the earliest diversification of extant lineages of Symbiodiniaceae occurred during the mid-Jurassic period. This timing coincides with a second and much larger adaptive radiation of modern shallow water corals (Stanley and Fautin 2001; Simpson et al. 2011; LaJeunesse et al. 2018). These endosymbioses have shaped coral holobiont biology by giving rise to specific adaptations, as evident, for example, the mode of intracellular reproduction and

metabolism of the symbiont (Fitt and Trench 1983; Liu et al. 2018) and the host's ability to use translocated photosynthates to meet most of its energy requirements and exhibit photosynthesis-enhanced calcification (Muscatine and Porter 1977; Falkowski et al. 1984). Some level of specificity between both entities exists (Trench 1993), which combined with the differential capacity to use light energy by the symbiont, creates niche diversification and largely explains vertical distribution in corals (Iglesias-Prieto et al. 2004).

Coral reefs are among the most diverse ecosystems providing important economic revenue through fisheries, tourism and pharmacology industries, and they also have a roll protecting seashores from erosion (Moberg and Folke 1999). However, coral reef ecosystems thrive under a relatively narrow temperature range (Buddemeier and Kinzie 1976; VeronQuery 1995), and when local temperatures surpass the normal average in conjunction to high light exposure, disruption of the symbioses by exocytosis and loss of light harvesting pigments occur (Weston et al. 2015), leading to coral bleaching (Glynn 1993, 1996; Goreau and Hayes 1994; Fitt et al. 2001). This process can be reversible or catastrophic depending on the geographic location, extent and duration of the perturbation (Boylan and Kleypas 2008; Donner 2011; Sully et al. 2019). It is predicted that more frequent and severe thermal anomalies will occur due to anthropogenic climate change (Spalding and Brown 2015). Therefore, it is imperative to better understand the potential plasticity and variability of the key biological traits of these symbioses under such thermal stress scenarios.

Based on early morphological studies *in hospite*, it was believed that Symbiodiniaceae algae isolated from different hosts were the same species, i.e., *Symbiodinium* (= *Gymnodinium*) *microadriaticum* Freudenthal (Taylor 1969, 1974). Other studies using a combination of biochemical and morphological characterisation of cultured Symbiodiniaceae found intrinsic differences of genetic origin (Schoenberg and Trench 1980a, b, c). The incongruence of *in hospit*e and *in vitro* studies along with the relative slow-developing classical morphological descriptions and some difficulties to culture some symbionts led to the development of gene-based approaches using the nuclear Small Subunit ribosomal DNA (SSU rDNA) to characterise the diversity and evolution of Symbiodiniaceae (Rowan and Powers 1991a, b, 1992). These studies showed the existence of a vast genetic diversity, and the existence of divergent evolutionary lineages (Clades A–C, now expanded to up to twelve different genera and clades in the family Symbiodiniaceae (LaJeunesse et al. 2018)). This genetic diversity was potentially linked to different physiology, later demonstrated by the differential response to light intensity of different cultured symbionts (Iglesias-Prieto and Trench 1994, 1997). Since this initial SSU rDNA characterisation, there have been a number of different approaches to elucidate the Symbiodiniaceae genetic attributes by using sequence-based methods using other markers such as the internal transcribed spacer or ITS regions of the ribosomal cistron and chloroplast non-coding regions of the psbA^{ncr} gene, coding for de D1 protein involved in photosynthesis (Zurawski et al. 1982; LaJeunesse 2001; LaJeunesse and Thornhill 2011), and sequence-independent techniques such as amplified fragment length polymorphism or AFLP and allozyme electrophoretic patterns (Coffroth et al. 1992; Baillie et al. 1998). The ITS2 marker has been most frequently used and more recently in high throughput sequencing aimed at detecting cryptic symbiont populations as well as improving on the discriminating power for those intragenomic variants of the tandemly repeated ribosomal DNA (Arif et al. 2014; Quigley et al. 2014; Cunning et al. 2017). There are also population studies using polymorphic microsatellites (Thornhill et al. 2017). Along with this uncovered genetic diversity, there is an extensive body of literature showing that physiological diversity among symbiont types is also large, both among members of the same as well as different genera (reviewed in (Quigley et al. 2018)). In line with this, to have a point of comparison with other related protozoa, the expression of distinct phenotypes by the same genotype is a commonly recognised phenomenon among free-living eukaryotic dinoflagellates, with potential implications for growth (Doblin et al. 2000), but may have further implications within the coral holobiont.

In the coral reef community, the Symbiodiniaceae taxa harboured by a coral colony have commonly been interpreted as a measure of its environmental stress tolerance, with physiological variation within types being largely ignored (Rowan 2004; Berkelmans and van Oppen 2006). However, a recent study has demonstrated that large differences in thermal tolerance can exist between allopatric populations of the same symbiodiniacean species (related to *Cladocopium goreaui,* and referred here as *Cladocopium* C1^{acro}), both *in vitro* and *in hospite*, a result of adaptation of these populations to their local environmental conditions (Howells et al. 2012; Levin et al. 2016). Here, we provide evidence for the existence of variation in the Symbiodiniaceae response to temperature at an even smaller spatial scale, among *Cladocopium* symbionts extracted from conspecific and sympatric hosts. We successfully adapted a non-sequence-based approach (TE-AFLP; Three Endonuclease Amplified Fragment Length Polymorphism) (van der Wurff et al. 2000) to distinguish individual isolates, with promising future applications for population-based studies of reef ecosystems. Our results highlight that a large portion of the physiological variation present within the Symbiodiniaceae is undocumented and mostly overlooked,

and that more caution is required when interpreting species/strain identity based on DNA sequence data of one or a small number of loci in the context of physiological performance.

Methods

Establishment of clonal cultures

Cladocopium C1^{acro} cells were isolated from four coral colonies growing at similar depths and photic habitats from the Central Great Barrier Reef (GBR): two *Acropora tenuis* ($\sim$ 3–4 m depth) and one *Acropora millepora* ($\sim$ 3 m depth) from Nelly Bay, Magnetic Island, and one *A. tenuis* ($\sim$ 3 m depth) from South Molle Island in the Whitsunday Islands (collected under the permit G10-33440.1). Host skeletons as well as living cells are kept at Symbiont Culture Facility, part of the Australian Institute of Marine Science, AIMS. Before the culturing of the symbionts, freshly isolated cells from the host were genotyped. These results were latter contrasted with the cultured dinoflagellates being both made of the same genetic makeup. Isolation of *Cladocopium* cells was carried out by air brushing the coral tissue off the skeleton, centrifugation (5 min at 1600 g) and resuspension of the pellet in 0.2 µm filtered seawater. Centrifugation and resuspension were repeated three times to remove organic debris. Symbiont cells were inoculated into sterile IMK growing media (Wako Chemicals USA, Richmond) with antibiotics (penicillin, neomycin, streptomycin, nystatin, final concentration [100 µg mL^{-1}] each, amphotericin final [2.5 µg mL^{-1}], plus GeO_2 final [50 µM] (Beltran et al. 2012). Cultures were maintained in 24-well plates at 26 °C, 60 $\pm$ 10 µmol quanta m^{-2} s^{-1} of photosynthetically active radiation (PAR) (light coming from the bottom), 14:10 light: dark photoperiod inside environmental chambers (Steridium e500). After a month of growing in vitro, only those cultures showing no signs of contamination were pooled and reinoculated in antibiotic-rich media or IMK+. Cells were allowed to grow for one more month before transferring them four more times into fresh IMK+ (total of five months) to minimize bacterial contamination. After this antibiotic treatment, cultures were expanded in volumes of 50 mL antibiotic-free media or IMK −, considering this the source population to establish clonal strains. For the generation of clonal cultures, two methods were used. Three of the four clonal cultures (Aten-MI-1, Aten-WSY, and Amil-MI) were obtained after plating the heterogeneous population onto IMK − 1% agar plus antibiotics and incubating them under the conditions as above and picking a single colony-forming unit. The fourth clonal culture (Aten-MI-2) was produced from a single cell isolated using a micro-manipulator (MN-153, Narishige) under an inverted microscope at 20X magnification (Leica DM IRB/E, Germany).

Genetic characterisation of Symbiodiniaceae cultures

For genotyping, DNA was extracted from exponentially growing cultures (10 mL culture centrifuged as above after which the culture medium was removed) using the Qiagen Plant DNA kit following manufacturer's protocol. Single-strand conformation polymorphism (SSCP) analysis of the ITS1 region was carried out following the protocol described in van Oppen et al. (2001), and direct sequencing using the dideoxy chain terminator method (Sanger et al. 1977) of the ITS2 and psbA^{ncr} regions following Stat et al. (2013) and LaJeunesse and Thornhill (2011), respectively. Sequence data from the chloroplast marker were deposited at the genbank with the accession numbers MW691103-06 for Aten-MI-2, Aten-MI-1, Amil-MI and Aten-WSY, respectively. A phylogenetic analysis of the psbA^{ncr} data was conducted using PAUP employing the maximum likelihood algorithm. The cultures were also characterised by TE-AFLP, (van der Wurff et al. 2000) and their products were analysed using GeneMarker Ver 2.6.4 and R studio Ver. 1.2.1.3 for WARD and UPGMA grouping algorithms. We chose these approaches, the first ones to distinguish at the genus and species level, and the last one to be able to distinguish at the individual level (for the latter, assuming asexual reproduction after clonal isolation).

Growth kinetics

The ratchet approach to compare the strains was chosen because we were interested on examining the differences in response to selection. For each strain, 16 falcon flat bottom tubes (50 mL) with a gas permeable PTFE membrane cap (Bioreactor 50, TPP) were inoculated with 2×10^5 cell mL^{-1} in a total volume of 30 mL. The tubes were incubated upright, light coming from the bottom at 60 $\pm$ 10 µmol quanta m^{-2} s^{-1} of PAR (Sylvania FHO24W/T5/865 fluorescent tubes) and a 14:10 light: dark photoperiod and 26 °C. To estimate growth, cultures were subsampled (4 replicates of 50 µL per tube) at regular intervals and cell density assessed by measuring absorbance at 750 nm (Pomati et al. 2003) using a plate reader (PowerWave, Bio-Tek). Absorbance was transformed to cell density using a calibration equation per strain (Aten-MI-1 = 13389863.995*OD @ 750 nm, Aten-MI-2 = 8452519.023*OD @ 750 nm, Aten-WSY = 14325213.125*OD @ 750 nm, Amil-MI = 9016121.922*OD @ 750 nm). After approximately 30

days of incubation, the four replicates of each strain showing the highest growth rate were selected for further experimentation. Cells from each replicate were equally divided over 16 tubes and cell densities adjusted as above. These fast 26 °C growing cultures were grown/sampled for the same period of time at 28 °C, selecting the fastest replicates, splitting them in 16 tubes, density adjusted to 2×10^5 cell mL^{-1} then incubated for a month at 30 °C, repeating the procedure mentioned before incubating at 32 °C the best growing cultures and for those surviving this last temperature, the final incubation growth was 34 °C, with lighting and photoperiod conditions in all "ratchet" steps as above. It is worth mentioning that for the last incubation temperatures, the replicates number were reduced to 8–10 tubes due to the poor growth observed under these stressful temperatures. These experiments were repeated three independent times from May to November of 2011, beginning with a one month old 26 °C culture as starter for the temperature ratcheting. Growth rate was calculated by least-squares fit of a straight line to the data (cell density of four consecutive sampling days), logarithmically transformed, based on the formula: μ = Ln (N1/N0)/t1 − t0 where μ is the growth rate in cell divisions * day^{-1}, and N0, N1 represent initial and final density of cells at t0 and t1, initial and final time, respectively (Guillard 1973). Because temperature could affect the growth kinetics, in our experiment we used two time windows to better characterise the timing of exponential growth. We called phase 1 and phase 2 from day 0 to day 11 (day 0, 3, 6, and 11) and from day 11 to day 18 (day 11, 13, 14, and 18) post inoculation, respectively. Growth rates were statistically compared using two-way ANOVA (temperature and phase), and the post-hoc Tukey test using the R-studio software. After Tukey test, the data generated were used to calculate the heterogeneity values by normalising with all six possible different pair combinations as a 100%.

Stability of thylakoid membranes

For the quantification of the fluidity of the thylakoid membranes, we followed the protocol of Diaz-Almeyda et al. (2011). Cultures growing exponentially at 26 °C and 31 °C (strait from 26 to 31 °C, with one month of acclimation) for one month under the conditions mentioned above were harvested by centrifugation at 1600 g for 5 min and resuspended in fresh sterile IMK − media to a density of 3×10^6 cell mL^{-1}. In addition to the four *Cladocopium* C1^{acro} cultures we included a heterogeneous culture of *Durusdinium trenchii* (formerly D1a) (isolated from *Acropora muricata* at Magnetic Island) to benchmark results with a phylogenetically distinct symbiont species. All cultures were dark adapted for 10 h at their growth temperature (either 26 °C or 31 °C) prior to the measurements. Six replicates of 100 µL each per culture per temperature were tested three independent times. Using a thermocycler (PTC-100, MJ Research), culture aliquots were exposed to 1 °C stepwise temperature increments (5 min each) from 32 to 43 °C under darkness. After the incubation period, maximum quantum yield, Fv/Fm of photosystem II, calculated according to Schreiber et al. (1994) and Warner et al. (1996), was measured (thermocycler heating block within the chamber of the fluorometer) using an imaging-PAM (Maxi-PAM, Walz Gmbh, Effeltrich, Germany). The Fv/Fm in response to temperature measures the uncoupling of energy transfer from the antennae complex to the reaction center as an indication of melting of the chloroplast membrane (Schreiber and Armond 1978). Differences in melting points among strains were statistically compared using a one-way ANOVA, followed by the post-hoc Tukey test using the NCSS software.

Results

Growth kinetics

Temperature-dependent growth performance for each strain shows a progressive decline, in response to temperature increments, as shown by the total biomass towards the end (23–30 days post inoculation) of the incubation period. Aten-MI-1 showed between 33 and 55% more biomass in comparison with the other three strains (Fig. 1). As growth rates were clearly affected by temperature, we decided to analyse in detail the window of time where exponential growth took place. Growth curves of the four clonal strains showed two distinct phases. The first was observed during the first 11 days following inoculation into fresh media, and the second was from day 11 to 18 (Fig. 1, dark and light shaded areas, respectively). By dividing in two windows of time, late and early growth rate in response to temperature can be seen, but also when there were no differences between these two phases we can conclude that for particular temperature the strain shows an extended maximum growth rate meaning that temperature does not have an effect on the timing and duration of growth (see for example Aten-MI-2 and Aten-WSY for the temperatures 26–30 °C, Fig. 2). Maximum growth rates were achieved in either the first or the second phase depending on strain and temperature. For instance, Aten-MI-1 and Amil-MI at 26 °C and 28 °C achieved their exponential growth in the first phase, while Aten-WSY and Aten-MI-2 at 30 °C reached their maximum growth rate in the second phase (Fig. 1). Temperature-dependent growth data were adjusted to different polynomial equations depending on the

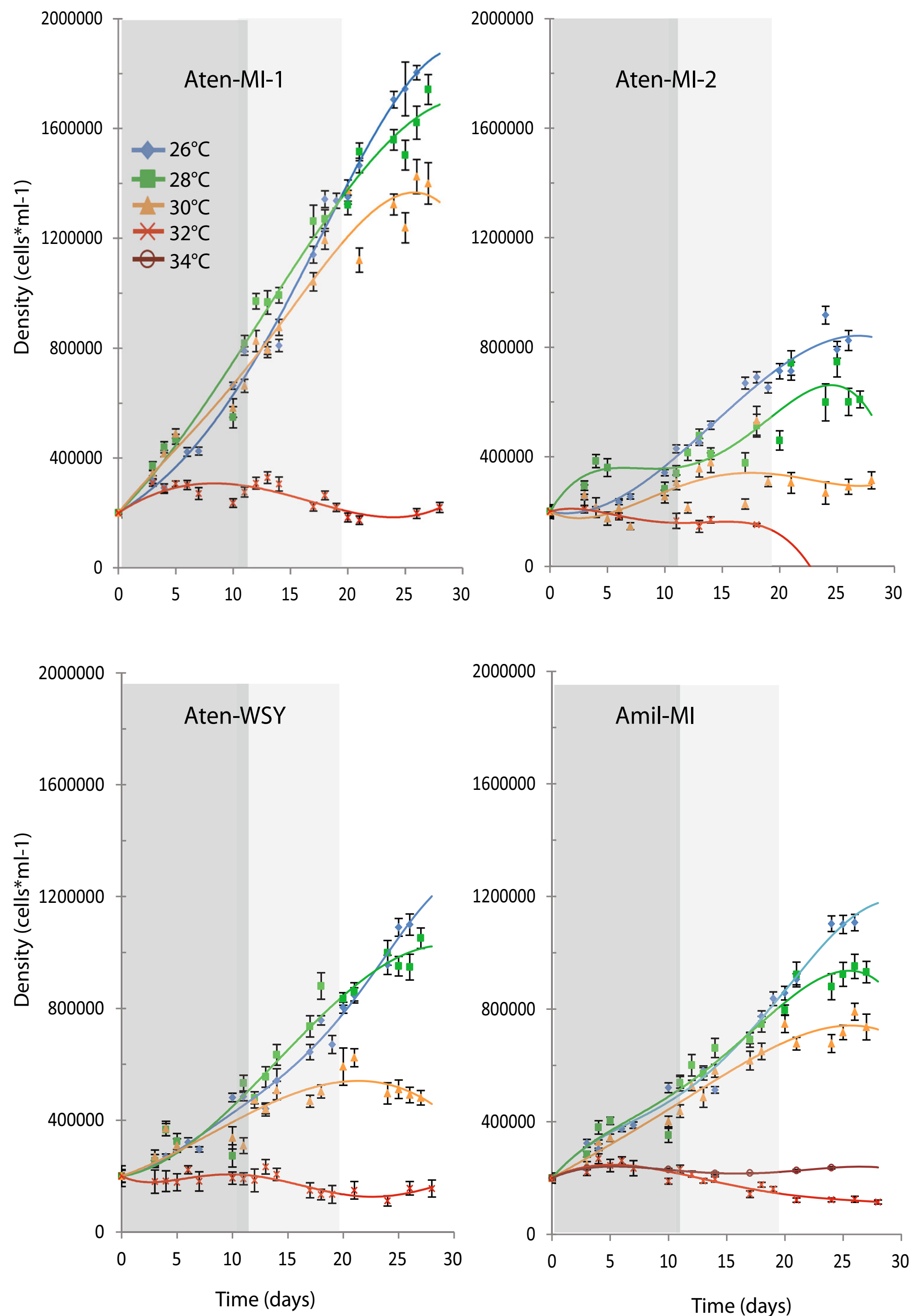

Fig. 1 Growth kinetics of four monoclonal *Cladocopium* C1^{acro} strains at five different temperatures (mean ± SD). Temperature treatments were consecutive, based on a ratchet design (see methods). The shaded areas represent the two windows of time or phases (dark and light gray for phase 1 and 2 respectively) analysed in this study. Growing equations and correlation index per each curve are depicted in Table 3

high coefficient value observed. These equations describe the growth behaviour (Fig. 1, coloured lines as best fit model) at different temperatures (Table 3).

Growth behaviour differed significantly among strains (Fig. 2, Table 1). In general, during phase 1 at 26 °C, we observe the highest heterogeneity (all six possible strain-growth combinations were different) in growth rate among the strains, with Aten-MI-1 being the strain with the highest growth rate (Fig. 2, comparative matrix under the main chart). High heterogeneity of 83% was also observed during the second phase at 32 °C followed by 67% observed during the 2nd and 1st phases at 26 °C and 28 °C, respectively. The less variable, or where most of the strains were “similar” to each other were the combination of temperature and time observed towards the end, during the

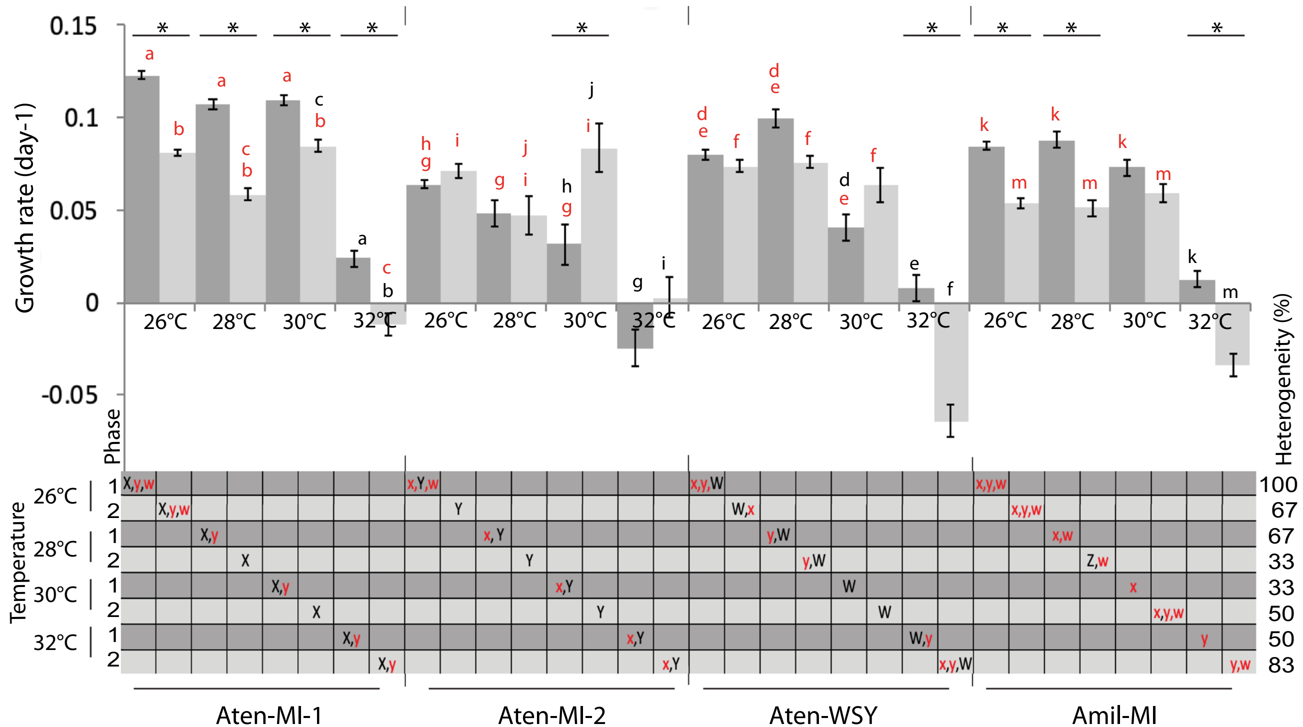

Fig. 2 Growth rate comparison among *Cladocopium* C1[acro] strains at two different time points (mean ± SD). Dark and light bars represent phase 1 (0–11 days post inoculation) and phase 2 (11–18 days post inoculation), respectively. Significant differences (see Table 1) are depicted as asterisks and letters (red coloured letters against its counterpart in black coloration), representing statistically significant differences between two phases at one given temperature per strain, and differences among all growing temperatures for a particular strain (top of the bars) and differences per phase and temperature among strains (matrix below the chart), respectively

Table 1 Two-way ANOVA results. Temperature and time (phase) effects in the growth rate "μ" (day-1) of the different symbiont strains

	Sum of squares	*df*	*F* value	*p* value
Temperature	1.7553	3	379.425	< 2.2e−16
Phase	0.171	1	110.918	< 2.2e−16
Temperature: Phase	0.0653	3	14.116	4.399e−09
Residuals	2.479	1608		

second phase at 28 °C and the first phase at 30 °C (Fig. 2, matrix below the chart).

When comparing within strains, in none of the cases, the growth observed in both phases at 26 °C and 28 °C was different (Fig. 2, chart). This low variability extends further at 30 °C in Amil-MI. The last temperature of 32 °C was the most variable within strain for all strains.

At the temporal scale, at each temperature, we found that Aten-MI-2 and Aten-WSY showed no differences between phases at 26 °C and 28 °C and the same was observed for the pair Amil-MI and Aten-WSY at 30 °C. This means these strains should have an extended exponential growth phase compared with Aten-MI-1 and Amil-MI for the same temperatures (Fig. 2, asterisk above bars).

Finally, it is worth noticing that the strain Amil-MI was the only one capable to survive long enough at 32 °C to be able to be transferred to 34 °C where there were no dramatic increases in biomass, but the cells did not die during the 30 day incubation test (Fig. 1, dark carmine line).

Stability of the photosynthetic membranes

The stability of chloroplast thylakoid membranes was assessed by pulse amplitude modulation (PAM) fluorometry of cultures maintained at 26 °C and acclimated to 26 °C and 31 °C for one month. All strains showed evidence of an acclimation response, as the melting of chloroplast thylakoid membranes were significantly higher in cultures maintained at 31 °C compared to the 26 °C (see

Table 2 One-way ANOVA results. Chloroplast melting temperature comparison between different symbiont strains

	Sum of squares	d*f*	*F* value	*p* value
Melting temperature	6.03E−02	9	93.36	< 1.0e−06
Error	7.90E−03	110		
Total	6.8225	119		

Table 2). Melting temperatures increased by 5.3% for the strains Aten-MI-1 and Aten-WSY and by 3.1% for the strains Aten-MI-2 and Amil-MI. Melting temperature change was the lowest in the *D. trenchii* strain with only 1.72% increase (Table 3). It is worth mentioning that two distinct patterns of acclimation were observed in symbionts isolated from the same reef and conspecific species (Fig. 3, see data for Aten-MI-1 vs. Aten-MI-2).

Genetic characterisation

The four *Cladocopuim* C1^{acro} strains showed identical ITS1 SSCP profiles (not shown) as well as an identical ITS2 sequence, which had 100% identity to *Cladocopium goreaui* (GenBank accession number AB778664.1). In line with this, there are at least 3 different lineages of *Cladocopium* from the GBR diagnosed by the sample ITS2 C1 sequence that are not *C. goreaui*, whose ribosomal array is also dominated by this same sequence variant. The four cultured strains belong to just one of these species lineages (T. LaJeunesse, personal communication, December, 2020). As for validating that all of our strains were actually one species, the phylogenetic analysis using the psbA^{ncr} marker clearly shows two distinctive clusters (Fig. 4a), one formed by sequences derived from *C. goreaui* isolated from Caribbean invertebrates, and the other formed with data from this communication along with other sequences from invertebrates sampled at the central GBR, proving we were dealing with a single undescribed *Cladocopium* species. This latter result justifies the validity of our DNA-fingerprinting approach using TE-AFLP in order to discriminate single genotypes. TE-AFLP analysis demonstrated that the four cultures represent distinct genotypes with an approximate 24% of shared bands (from a total 135 scored bands) below the 150 bp size mark. The analysis grouped the coral symbionts at between 77 and 84% of similarity (calculated using UPGMA. WARD algorithm also rendered same groups with similar distance among them) (Fig. 4b). The overall level of similarity of all strains was 65% (Fig. 4b, dendrogram). From a total of 135 events registered (bands of different sizes) between 60 to 460 bp, there was a total and average variation of 39.2 and 17.6%, respectively. The strains Aten-WSY and Aten-MI-2 showed more large sized (over 350 bp) fragments compared to the other two (Fig. 4b, see chart below the dendrogram).

Discussion

Growth rate

The coral holobiont thrives under a narrow temperature range, which is why we are interested to understand the relationship of temperature and growth of the photosynthetic partner of this symbiosis. Recent Symbiodiniaceae genome data, including for the strain Aten-MI-1, points to positive selection for genes with functions related to photosynthesis, possibly selecting to increase thermal resilience (Liu et al. 2018), confirming an important fitness measure are growth rate in relation to temperature. Members of the Symbiodiniaceae display *in hospite* growth rates spanning one order of magnitude from 0.1 to 0.01 d^{-1}. These rates are lower compared to growth rates of many planktonic dinoflagellates (Wilkerson et al. 1983). As for temperature effects in cultured symbionts, distantly related *Symbiodinium microadriaticum* (type A1) at 26 °C and *Effrenium voratum* (clade E) (previously known as *S. californium* (Jeong et al. 2014)) at 16 °C showed highly similar growth rates of 0.199 d^{-1} and 0.196 d^{-1}, respectively (Banaszak and Trench 1995). Conversely, different growth rates at the same temperature of 25 °C were measured in members of the genus *Brevolium* (clade B) and *Fugacium* (clade F) (0.40 d^{-1} and 0.55 d^{-1}, respectively (Fuchinoue et al. 2012)), and similar values of 0.25 d^{-1} and 0.20 d^{-1} were recorded for *S. pilosum* (type A2) and *Fugacium kawagutti* (type F1), respectively (Rodriguez-Roman and Iglesias-Prieto 2005). In regards to *Cladocopium* (clade C), a highly diverse genus (LaJeunesse et al. 2018), also known to associate with a number of invertebrates including scleractinian corals (Yuyama and Higuchi 2014), temperature-dependent growth observed in symbionts belonging to this genus showed a decline in kinetics towards temperatures above 30 °C (Karim et al. 2015; Grégoire et al. 2017) similar to our results (Fig. 2), however, with a different magnitude, potentially explained because they likely represent different genotypes and were isolated from molluscs and not corals. Intraspecific strain variation in growth rate have been observed in *Breviolum*

Table 3 Temperature dependent growing equations of four strains of *Cladocopium* C1[acro], where "y" represents the density in cells per millilitre at a given "x" time

Strain\temperature	26 °C	28 °C	30 °C	32 °C	34 °C
Aten-MI-1	$y = -3.5805x^4 + 111.83x^3 + 846.81x^2 + 26957x + 200{,}000$	$y = -0.462x^4 - 34.426x^3 + 1738.4x^2 + 41571x + 200{,}000$	$y = -4.7583x^4 + 187.46x^3 - 1939.1x^2 + 52196x + 200{,}000$	$y = 0.8572x^4 + 9.1927x^3 - 1888.8x^2 + 27540x + 200{,}000$	No data
	$R^2 = 0.9915$	*$R^2 = 0.9781$*	*$R^2 = 0.9638$*	*$R^2 = 0.6541$*	
Aten-MI-2	$y = -78.713x^3 + 3338.6x^2 - 8984x + 200{,}000$	$y = -12.246x^4 + 653.96x^3 - 10771x^2 + 70262x + 200{,}000$	$y = 4.3378x^4 - 272.01x^3 + 5079.1x^2 - 20761x + 200{,}000$	$y = -9.6056x^4 + 369.07x^3 - 4373.6x^2 + 12491x + 200{,}000$	No data
	$R^2 = 0.9751$	*$R^2 = 0.8103$*	*$R^2 = 0.411$*	*$R^2 = 0.9008$*	
Aten-WSY	$y = -0.2843x^5 + 19.111x^4 - 438.15x^3 + 4690.2x^2 + 3159.5x + 200{,}000$	$y = -61.107x^3 + 2570.6x^2 + 5283.3x + 200{,}000$	$y = -40.095x^3 + 962.89x^2 + 13670x + 200{,}000$	$y = -0.2162x^5 + 18.099x^4 - 513.01x^3 + 5577x^2 - 19819x + 200{,}000$	No data
	$R^2 = 0.9826$	*$R^2 = 0.9486$*	*$R^2 = 0.8038$*	*$R^2 = 0.7089$*	
Amil-MI	$y = -5.8291x^4 + 314.1x^3 - 4715.8x^2 + 48625x + 200{,}000$	$y = -5.7492x^4 + 277.31x^3 - 4067x^2 + 47599x + 200{,}000$	$y = -1.1316x^4 + 29.814x^3 - 121.93x^2 + 23728x + 200{,}000$	$y = -1.3863x^4 + 108.26x^3 - 2798x^2 + 20867x + 200{,}000$	$y = -2.0198x^4 + 128.57x^3 - 2621.9x^2 + 18314x + 200{,}000$
	$R^2 = 0.9878$	*$R^2 = 0.9621$*	*$R^2 = 0.9643$*	*$R^2 = 0.9014$*	*$R^2 = 0.9955$*

Italic values are the correlation coefficient

psygmophilum strains, with an average growth of 0.31 d^{-1} under 12:12 light photoperiod, 25 °C and 60 µmol quanta m^{-2} s^{-1} of PAR (Parkinson and Baums 2014), and also responses under a variety of temperatures has recently been documented for *S. microadriaticum* (Diaz-Almeyda et al. 2017; Klueter et al. 2017). Similarly, our growth rate estimate for the strain Aten-MI-1 at 26 °C was 0.12 d^{-1} (Fig. 2), compared with 0.16 d^{-1} (Chakravarti et al. 2017), and 0.17 d^{-1} for the original heterogeneous C1 culture (Raina et al. 2017), both data taken at 27 °C. When exposed to a relatively high temperature above 30 °C, our results showed a less dramatic decline in growth, assuming an average between both phases to compare with densities from day 3 and 17 used in Chakravarti et al. (2017). These deviations can be explained in terms of timing of measurements and potential acclimation to different conditions *in vitro*. Our research is the closest to the isolation time from the coral host and potentially more likely to resemble the actual growth rate *in hospite*. However, Aten-MI-1 along with our other three strains are part of a broad symbiont collection isolated from a number of hosts from the GBR and the incubation conditions were standardised for all of them. The combination of changes in incubation temperature and potential acclimatory/adaptive effects due to the elapsed time growing *in vitro* may have affected the deviations observed in growth rates.

The two cultures that were isolated from different but conspecific coral colonies (*A. tenuis*) growing on the same reef (Nelly Bay, Magnetic Island) and in the similar photic habitat ($\sim$ 3–4 m depth), show remarkable differences in growth behaviour and thylakoid membrane fluidity (Figs. 1, 2, 3). We hypothesise that previous history along with micro-ambient and genetic differences could explain the observed behaviour. This level of variation was previously described for conspecific coral and its algal symbionts (heterogeneous cultures) isolated from different geographical locations with different temperature regimes (Howells et al. 2012). In our research, clonal strains derived from these two heterogeneous cultures were still showing differences in thylakoid membrane fluidity (Fig. 3, see Aten-MI-2 vs. Aten-WSY). Remarkably, the response of an apparently cryptic symbiont, Amil-MI matches one of the two well-observed behaviours. These symbionts were discovered by chance because the coral colony from which they were originally isolated was genotyped as type D1a (VHB, unpublished data), in agreement with a previous report for this coral species in this location (Little et al. 2004).

Photosynthetic response to elevated temperatures

In the same way, we characterised growth kinetics under different temperatures, we wanted to extend our

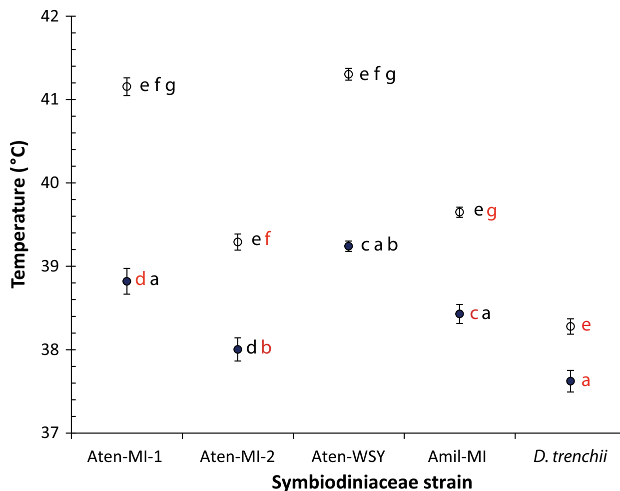

Fig. 3 Melting temperatures (mean ± SD) of the photosynthetic membranes of five strains of Symbiodiniaceae cultures after having been grown at 26 °C (closed symbols) and at 31 °C (open symbols) for 30 days. Significant differences (see Table 2) are depicted beside the symbols with red coloured letters against its counterpart in black colouration

understanding of the behaviour of the photosynthetic apparatus under thermal stress. Loss of photosynthetic activity due to thermal stress has been documented for a number of primary producers, including Symbiodiniaceae (Iglesias-Prieto et al. 1992; Sato et al. 1996; Inoue et al. 2001). Our *Cladocopium* C1^{acro} clonal strains growing at 26°C showed a considerable level of variation (up to 1.24 °C) in the decrease of 50% of maximum quantum yield (Fig. 3, see pair Aten-MI-2 & Aten-WSY, for example). Similar responses were observed by Diaz-Almeyda et al. (2011) between *Cladocopium goreaui* and the distantly related *Symbiodinium pilosum* (type A2) strains, with melting values of 36.7 °C and 37.7 °C, respectively. The values observed by these authors for their *C. goreaui* strain were 1.7 °C lower than the average (38.4 °C) for the four clonal symbiont cultures in our study. Diaz-Almeyda et al. (2011) used a strain of *C. goreaui* and therefore representative of a different species from ours (T. LaJeunesse, personal communication, December 2020), also the fact that they used a lower growing temperature (24 °C instead of the 26 °C employed in our experiment) may have contributed to the difference in results. The difference between the melting temperature of the thylakoid membranes and the growing temperature was very similar in both studies (taking the average of the four strains in this study), with a value of 12.5 °C. When cultures were grown at 31 °C, the averaged melting value of our four clonal *Cladocopium* cultures was 39.9 °C and very close to that reported for *C. goreaui* by Diaz-Almeyda et al. (2011) of 40.2 °C. This increase in thermal tolerance after a period of acclimation has also been observed for other Symbiodiniaceae species in a similar experimental setting (Takahashi et al. 2013). Despite these similarities with other studies, we observed significant differences in the acclimation capacity as well as the absolute thylakoid membrane melting temperatures among the four *Cladocopium* strains (Fig. 3, Table 2), with the difference being of the same order of magnitude to those observed between different genera (Diaz-Almeyda et al. 2011). These observations highlight the physiological diversity among sympatric genotypes of the same Symbiodiniaceae species as observed based on their growth behaviour.

In our experiment, we used *Durusdinium trenchii* as a point of comparison for the *Cladocopium* C1^{acro} clonal strains. In line with this, the genus *Durusdinium* has been typically reported as being thermotolerant (Glynn et al. 2001, Baker et al. 2004, Rowan 2004, Ulstrup et al. 2006), and in particular *D. trenchii* (LaJeunesse et al. 2009, Hoadley et al. 2019). This culture showed a lower melting point compared with the clonal *Cladocopium* strains (Fig. 3). *D. trenchii* strain, however, showed a faster recovery rate after thermal stress compared with heterogeneous *Cladocopium* C1^{acro} culture (VHB, unpublished data). In line with this, it is been observed that *Cladocopium* C1^{acro} confers the holobiont with higher thermotolerance than *D. trenchii* symbionts in *Acropora tenuis* stressing the importance of the host-symbiont interaction (Abrego et al. 2008). Finally, the observed differences between species could be explained by different regulatory mechanisms altering composition of fatty acids and antioxidant systems which seem to control the extent of acclimation and response to thermal stress (Tchernov et al. 2004, Diaz-Almeyda et al. 2011, Krueger et al. 2014).

Genetic identification of Symbiodiniaceae

The field of taxonomic characterisation of the members of the Symbiodiniaceae family has been in part impaired due to the intrinsic "coccoid" morphology *in hospite*, this along with some uncultivable isolates (Goulet and Coffroth 2003) witness the birth of biochemical and genetic characterisation (Schoenberg and Trench 1980a, b, c; Rowan and Powers 1991b; Baillie et al. 1998). The fact that our strains behaved differently (see Figs. 1 and 3) while having the same ITS1 SSCP profile and ITS-2 sequence led us to adopt a DNA fingerprinting similar to the AFLP, previously used in reef symbiotic invertebrates (Coffroth et al. 1992; Amar et al. 2008) but with the difference that the TE-AFLP uses three endonucleases instead of two in AFLP, with the combination of selective amplification that renders more discriminatory power with less number of bands (van der Wurff et al. 2000). Our results showed differences in their DNA fingerprint, forming two distinct clusters (Fig. 4b). Long or short (differences between growth

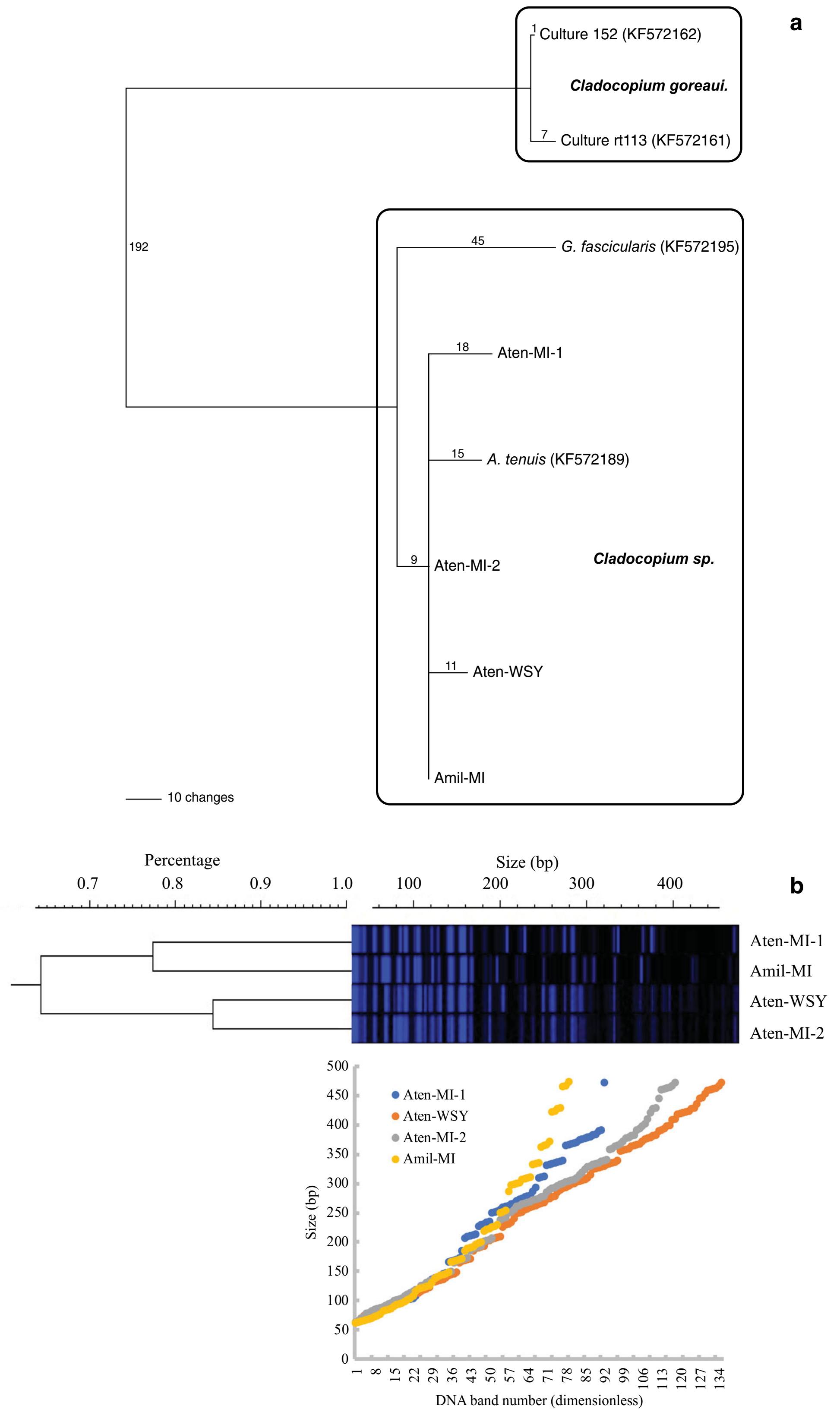

Fig. 4 Genetic identification of the symbionts. **a** Phylogenetic tree of the chloroplast psbA^{ncr} marker using maximum likelihood. The analysis includes some *C. goreaui* isolates along with *Cladocopium* C1^{cro}. Accession numbers for sequences not from this research are depicted in brackets. Accession numbers for the strains used in this research can be found in the methods section. **b** TE-AFLP analysis of the monoclonal *Cladocopium* C1^{acro} strains. Tree created using UPGMA showing percentage of similarity using the size window of 60–470 bp. The chart below represents the visual signature of the combination of the DNA band number as it appears in the electrophoresis and its molecular weight

phases) exponential growth periods (see Fig. 2) matched the clusters based on the TE-AFLP data; however, the chloroplast melting temperature data did not (i.e., Aten MI-2 vs. Aten-WSY in the TE-AFLP and Aten-WSY vs Aten MI-1 in the melting chloroplast experiment). This implies some level of caution when interpreting melting temperature data in the context of "relatedness" between genotypes and reassures the growing kinetics data as potentially more informative in terms of taxonomic similarities. The TE-AFLP approach has the advantage over ITS sequence analysis that this technology is the outcome of a large portion of the genome being analysed rather than a single locus, which has sometimes revealed an incongruence between genetic relatedness physiological response, for instance, the differential thermotolerance observed in closely related ITS2 types (Tchernov et al. 2004). We advocate other Symbiodininiaceae research to apply this method to characterise their cultures/isolates.

Implications of our findings

In the present communication, we demonstrated that different strains of *Cladocopium* C1^{acro} differ physiologically in response to temperature treatments. These differences in growth and photobiology likely influence the overall physiological tolerance among coral colonies living in heterogeneous environments. The combined inter-individual variation among genotypes of host and symbiont has important implications with regard to processes of natural selection and resilience to changing environmental conditions. Further investigations are needed to examine the breadth of physiological diversity created by the interactions of different host and symbiont genotype combinations.

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Declarations

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

References

Abrego D, Ulstrup KE, Willis BL, van Oppen MJH (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. Proceedings of the Royal Society B-Biological Sciences 275:2273–2282

Amar KO, Douek J, Rabinowitz C, Rinkevich B (2008) Employing of the amplified fragment length polymorphism (AFLP) methodology as an efficient population genetic tool for symbiotic cnidarians. Mar Biotechnol 10:350–357

Arif C, Daniels C, Bayer T, Banguera-Hinestroza E, Barbrook A, Howe CJ, Lajeunesse TC, Voolstra CR (2014) Assessing *Symbiodinium* diversity in scleractinian corals via next-generation sequencing-based genotyping of the ITS2 rDNA region. Molecular Ecology 23:4418–4433

Baillie B, Monje V, Silvestre V, Sison M, Belda-Baillie C (1998) Allozyme electrophoresis as a tool for distinguishing different zooxanthellae symbiotic with giant clams. Proceedings of the Royal Society of London Series B: Biological Sciences 265:1949–1956

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Corals' adaptive response to climate change. Nature 430:741–741

Banaszak AT, Trench RK (1995) Effects of ultraviolet (UV) radiation on marine microalgal-invertebrate symbioses.1. Response of the algal symbionts in culture and in hospite. Journal of Experimental Marine Biology and Ecology 194:213–232

Beltran VH, Dunlap WC, Long PF (2012) Comparison of the photosynthetic bleaching response of four coral species common to the central GBR. Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia, 9–13 July 2012 9A Coral bleaching and climate change:6pp

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a "nugget of hope" for coral reefs in an era of climate change. Proceedings of the Royal Society B-Biological Sciences 273:2305–2312

Boylan P, Kleypas J (2008) New insights into the exposure and sensitivity of coral reefs to ocean warming. Proc 11th Int Coral Reef Symp 2:854–858

Buddemeier R, Kinzie R (1976) Coral growth. Oceanogr Mar Biol Annu Rev 14:183–225

Chakravarti LJ, Beltran VH, van Oppen MJH (2017) Rapid thermal adaptation in photosymbionts of reef-building corals. Global Change Biology 23:4675–4688

Coffroth M, Lasker H, Diamond M, Bruenn J, Bermingham E (1992) DNA fingerprints of a gorgonian coral: A method for detecting clonal structure in a vegetative species. Mar Biol 114:317–325

Cunning R, Gates RD, Edmunds PJ (2017) Using high-throughput sequencing of ITS2 to describe Symbiodinium metacommunities in St. John US Virgin Islands. PeerJ 5:e3472

Diaz-Almeyda E, Thome PE, El Hafidi M, Iglesias-Prieto R (2011) Differential stability of photosynthetic membranes and fatty acid composition at elevated temperature in *Symbiodinium*. Coral Reefs 30:217–225

Diaz-Almeyda EM, Prada C, Ohdera AH, Moran H, Civitello DJ, Iglesias-Prieto R, Carlo TA, LaJeunesse TC, Medina M (2017) Intraspecific and interspecific variation in thermotolerance and photoacclimation in *Symbiodinium* dinoflagellates. Proceedings of the Royal Society B: Biological Sciences 284:20171767

Doblin MA, Blackburn SI, Hallegraeff GM (2000) Intraspecific variation in the selenium requirement of different geographic strains of the toxic dinoflagellate Gymnodinium catenatum. J Plankton Res 22:421–432

Donner SD (2011) An evaluation of the effect of recent temperature variability on the prediction of coral bleaching events. Ecological Applications 21:1718–1730

Falkowski PG, Dubinsky Z, Muscatine L, Porter WJ (1984) Light and bioenergetics of a symbiotic coral. Bioscience 34:705–709

Fitt WK, Trench RK (1983) The relation of diel patterns of cell division to diel patterns of motility in the symbiotic

dinoflagellate *Symbiodinium microadriaticum* Freudenthal in culture. New Phytol 94:421–432
Fitt WK, Brown BE, Warner ME, Dunne RP (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs 20:51–65
Fuchinoue Y, Katayama T, Obata M, Murata A, Kinzie R, Taguchi S (2012) Growth, biochemical properties, and chlorophyll fluorescence of symbiotic and free-living dinoflagellates in response to ammonium enrichment. Journal of Experimental Marine Biology and Ecology 438:1–6
Glynn PW (1993) Coral reef bleaching: ecological perspectives. Coral Reefs 12:1–17
Glynn PW (1996) Coral reef bleaching: Facts, hypotheses and implications. Global Change Biology 2:495–509
Glynn PW, Mate JL, Baker AC, Calderon MO (2001) Coral bleaching and mortality in panama and Ecuador during the 1997–1998 El Nino-Southern oscillation event: Spatial/temporal patterns and comparisons with the 1982–1983 event. Bulletin of Marine Science 69:79–109
Goreau TJ, Hayes RL (1994) Coral bleaching and ocean" hot spots". Ambio-Journal of Human Environment Research and Management 23:176–180
Goulet T, Coffroth M (2003) Genetic composition of zooxanthellae between and within colonies of the octocoral Plexaura kuna, based on small subunit rDNA and multilocus DNA fingerprinting. Marine Biology 142:233–239
Grégoire V, Schmacka F, Coffroth MA, Karsten U (2017) Photophysiological and thermal tolerance of various genotypes of the coral endosymbiont Symbiodinium sp. (Dinophyceae). Journal of Applied Phycology 29:1893–1905
Guillard RRL (1973) Division rates. In: Stein JR (ed) Handbook of phycological methods: culture and growth measurements. Cambridge University Press, Cambridge, pp 289–311
Hoadley KD, Lewis AM, Wham DC, Pettay DT, Grasso C, Smith R, Kemp DW, LaJeunesse TC, Warner ME (2019) Host–symbiont combinations dictate the photo-physiological response of reef-building corals to thermal stress. Sci Rep-Uk 9:1–15
Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2012) Coral thermal tolerance shaped by local adaptation of photosymbionts. Nature Climate Change 2:116–120
Iglesias-Prieto R, Trench RK (1994) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. Marine Ecology Progress Series 113:163–175
Iglesias-Prieto R, Trench RK (1997) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. II. response of chlorophyll-protein complexes to different photon-flux densities. Marine Biology 130(1):23–33
Iglesias-Prieto R, Matta JL, Robins WA, Trench RK (1992) Photosynthetic response to elevated temperature in the symbiotic dinoflagellate Symbiodinium microadriaticum in culture. Proceedings of the National Academy of Sciences U S A 89:10302–10305
Iglesias-Prieto R, Beltran VH, LaJeunesse TC, Reyes-Bonilla H, Thome PE (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. Proceedings of the Royal Society of London Series B-Biological Sciences 271:1757–1763
Inoue N, Taira Y, Emi T, Yamane Y, Kashino Y, Koike H, Satoh K (2001) Acclimation to the growth temperature and the high-temperature effects on photosystem II and plasma membranes in a mesophilic cyanobacterium, Synechocystis sp. PCC6803. Plant Cell Physiol 42:1140–1148
Jeong HJ, Lee SY, Kang NS, Yoo YD, Lim AS, Lee MJ, Kim HS, Yih W, Yamashita H, LaJeunesse TC (2014) Genetics and morphology characterize the dinoflagellate Symbiodinium voratum, n. sp., (Dinophyceae) as the sole representative of Symbiodinium clade E. Journal of Eukaryotic Microbiology 61:75–94
Karim W, Nakaema S, Hidaka M (2015) Temperature effects on the growth rates and photosynthetic activities of Symbiodinium cells. Journal of Marine Science and Engineering 3:368–381
Klueter A, Trapani J, Archer FI, McIlroy SE, Coffroth MA (2017) Comparative growth rates of cultured marine dinoflagellates in the genus Symbiodinium and the effects of temperature and light. PLoS ONE 12:e0187707
Krueger T, Becker S, Pontasch S, Dove S, Hoegh-Guldberg O, Leggat W, Fisher PL, Davy SK (2014) Antioxidant plasticity and thermal sensitivity in four types of *Symbiodinium* sp. J Phycol 50:1035–1047
LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: In search of a "species" level marker. J Phycol 37:866–880
LaJeunesse TC, Thornhill DJ (2011) Improved resolution of reef-coral endosymbiont (Symbiodinium) species diversity, ecology, and evolution through psbA non-coding region genotyping. PLoS ONE 6(12):e29013
LaJeunesse TC, Smith RT, Finney J, Oxenford H (2009) Outbreak and persistence of opportunistic symbiotic dinoflagellates during the 2005 Caribbean mass coral 'bleaching'event. Proceedings of the Royal Society B: Biological Sciences 276:4139–4148
LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr Biol 28(2570–2580):e2576
Levin RA, Beltran VH, Hill R, Kjelleberg S, McDougald D, Steinberg PD, Van Oppen MJ (2016) Sex, scavengers, and chaperones: transcriptome secrets of divergent Symbiodinium thermal tolerances. Molecular biology and evolution 33:2201–2215
Little AF, van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. Science 304:1492–1494
Liu H, Stephens TG, González-Pech RA, Beltran VH, Lapeyre B, Bongaerts P, Cooke I, Aranda M, Bourne DG, Forêt S (2018) *Symbiodinium* genomes reveal adaptive evolution of functions related to coral-dinoflagellate symbiosis. Commun Biol 1:95
Moberg F, Folke C (1999) Ecological goods and services of coral reef ecosystems. Ecol Econ 29:215–233
Muscatine L, Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27:454–460
Parkinson JE, Baums IB (2014) The extended phenotypes of marine symbioses: ecological and evolutionary consequences of intraspecific genetic diversity in coral–algal associations. Front Microbiol 5:445
Pomati F, Neilan BA, Suzuki T, Manarolla G, Rossetti C (2003) Enhancement of intracellular saxitoxin accumulation by lidocaine hydrochloride in the cyanobacterium Cylindrospermopsis raciborskii T3 (Nostocales). J Phycol 39:535–542
Quigley KM, Baker A, Coffroth M, Willis BL, van Oppen MJ (2018) Bleaching resistance and the role of algal endosymbiontsCoral bleaching. Springer, pp111-151
Quigley KM, Davies SW, Kenkel CD, Willis BL, Matz MV, Bay LK (2014) Deep-Sequencing Method for Quantifying Background Abundances of Symbiodinium Types: Exploring the Rare Symbiodinium Biosphere in Reef-Building Corals. Plos One 9
Raina JB, Clode PL, Cheong S, Bougoure J, Kilburn MR, Reeder A, Foret S, Stat M, Beltran V, Thomas-Hall P, Tapiolas D, Motti CM, Gong B, Pernice M, Marjo CE, Seymour JR, Willis BL, Bourne DG (2017) Subcellular tracking reveals the location of dimethylsulfoniopropionate in microalgae and visualises its uptake by marine bacteria. Elife 6

Rodriguez-Roman A, Iglesias-Prieto R (2005) Regulation of photochemical activity in cultured symbiotic dinoflagellates under nitrate limitation and deprivation. Marine Biology 146:1063–1073

Rowan R (2004) Coral bleaching - Thermal adaptation in reef coral symbionts. Nature 430:742–742

Rowan R, Powers DA (1991a) Molecular genetic identification of symbiotic dinoflagellates (zooxanthellae). Marine Ecology Progress Series 71:65–73

Rowan R, Powers DA (1991b) A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbioses. Science 251:1348–1351

Rowan R, Powers DA (1992) Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). Proc Natl Acad Sci U S A 89:3639–3643

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proceedings of the national academy of sciences 74:5463–5467

Sato N, Sonoike K, Kawaguchi A, Tsuzuki M (1996) Contribution of lowered unsaturation levels of chloroplast lipids to high temperature tolerance of photosynthesis in Chlamydomonas reinhardtii. Journal of Photochemistry and Photobiology B: Biology 36:333–337

Schoenberg DA, Trench RK (1980a) Genetic variation in *Symbiodinium* (= *Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. I. Isoenzyme and soluble protein patterns of axenic cultures of *Symbiodinium microadriaticum*. Proceedings of the Royal Society of London Series B: Biological Sciences 207:405–427

Schoenberg DA, Trench RK (1980b) Genetic variation in *Symbiodinium* (=*Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. II. Morphological variation in *Symbiodinium microadriaticum*. Proceedings of the Royal Society of London Series B: Biological Sciences 207:429–444

Schoenberg DA, Trench RK (1980c) Genetic variation in *Symbiodinium* (= *Gymnodinium*) *microadriaticum* Freudenthal, and specificity in its symbiosis with marine invertebrates. III. Specificity and inlfectivity of *Symbiodinium microadriaticum*. Proceedings of the Royal Society of London Series B: Biological Sciences 207:445–460

Schreiber U, Armond PA (1978) Heat-induced changes of chlorophyll fluorescence in isolated chloroplasts and related heat-damage at the pigment level. Biochem Biophys Acta 502:138–151

Schreiber U, Bilger W, Neubauer C (1994) Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of in vivo photosynthesis. In: Caldwell M (ed) Ecophysiology of photosynthesis (Ecological Studies, vol 100) Schilze, E. Springer, Berlin Heidelberg New York, pp 49–70

Simpson C, Kiessling W, Mewis H, Baron-Szabo RC, Müller J (2011) Evolutionary diversification of reef corals: a comparison of the molecular and fossil records. Evolution International Journal of Organic Evolution 65:3274–3284

Spalding M, Brown B (2015) Warm-water coral reefs and climate change. Science 350:769–771

Stanley GD, Fautin DG (2001) Paleontology and evolution - The origins of modern corals. Science 291:1913–1914

Stat M, Pochon X, Franklin EC, Bruno JF, Casey KS, Selig ER, Gates RD (2013) The distribution of the thermally tolerant symbiont lineage (Symbiodinium clade D) in corals from Hawaii: correlations with host and the history of ocean thermal stress. Ecology and Evolution 3:1317–1329

Sully S, Burkepile D, Donovan M, Hodgson G, Van Woesik R (2019) A global analysis of coral bleaching over the past two decades. Nat Commun 10:1–5

Takahashi S, Yoshioka-Nishimura M, Nanba D, Badger MR (2013) Thermal Acclimation of the Symbiotic Alga Symbiodinium spp. Alleviates Photobleaching under Heat Stress. Plant Physiol 161:477–485

Taylor DL (1969) Identity of zooxanthellae isolated from some Pacific Tridacnidae. J Phycol 5:336–340

Taylor DL (1974) Symbiotic marine algae: taxonomy and biological fitness. Symbiosis in the Sea, ed. W. B Vernberg. Columbia, SC: Univ. South Carolina Press: 245–262

Tchernov D, Gorbunov MY, de Vargas C, Yadav SN, Milligan AJ, Haggblom M, Falkowski PG (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proceedings of the National Academy of Sciences of the United States of America 101:13531–13535

Thornhill D, Howells E, Wham D, Steury T, Santos S (2017) Population genetics of reef coral endosymbionts (Symbiodinium, Dinophyceae). Molecular Ecology 26:2640–2659

Trench R (1993) Microalgal-invertebrate symbiosis, a review. Endocytobiosis Cell Res 9:135–175

Trench RK (1997) Diversity of symbiotic dinoflagellates and the evolution of microalgal-invertebrate symbioses. Proc 8th Int Coral Reef Symp 2:1275–1286

Ulstrup KE, Berkelmans R, Ralph PJ, van Oppen MJH (2006) Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae. Marine Ecology-Progress Series 314:135–148

van der Wurff AWG, Chan YL, van Straaien NM, Schouten J (2000) TE-AFLP: combining rapidity and robustness in DNA fingerprinting. Nucleic Acids Research 28:e105

van Oppen MJH, Palstra FP, Piquet AMT, Miller DJ (2001) Patterns of coral-dinoflagellate associations in Acropora: significance of local availability and physiology of Symbiodinium strains and host-symbiont selectivity. Proceedings of the Royal Society of London Series B-Biological Sciences 268:1759–1767

Veron JEN (1995) Corals in space and time: The biogeography and evolution of the Scleractinia. Ithaca, NY: Cornell University Press:321pp

Warner ME, Fitt WK, Schmidt GW (1996) The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in hospite from four different species of reef coral: A novel approach. Plant Cell Environ 19:291–299

Weston AJ, Dunlap WC, Beltran VH, Starcevic A, Hranueli D, Ward M, Long PF (2015) Proteomics links the redox state to calcium signaling during bleaching of the scleractinian coral Acropora microphthalma on exposure to high solar irradiance and thermal stress. Molecular & Cellular Proteomics 14:585–595

Wilkerson FP, Muller P, Muscatine L (1983) Temporal patterns of cell division in natural populations of endosymbiotic algae. Limnol Oceanogr 28:1009–1014

Yuyama I, Higuchi T (2014) Comparing the effects of symbiotic algae (Symbiodinium) clades C1 and D on early growth stages of Acropora tenuis. PLoS ONE 9:e98999

Zurawski G, Bohnert HJ, Whitfeld PR, Bottomley W (1982) Nucleotide sequence of the gene for the Mr 32,000 thylakoid membrane protein from Spinacia oleracea and Nicotiana debneyi predicts a totally conserved primary translation product of Mr 38,950. Proceedings of the National Academy of Sciences 79:7699–7703

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