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NOTE

Reduced Symbiodiniaceae diversity in *Palythoa tuberculosa* at a heavily acidified coral reef

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Abstract Symbiodiniaceae diversity in hosts is known to change with the environment and particularly with temperature and light intensity. However, higher levels of pCO_2, as could be expected under future ocean acidification scenarios, have been documented to show little to no effect in influencing the diversity of Symbiodiniaceae in hosts in previous studies. In this study, we examined hypervariable psbA^{ncr} sequences to identify the *Cladocopium* (former *Symbiodinium* 'Clade C') diversity within the zooxanthellate zoantharian *Palythoa tuberculosa* at an acidified reef in southern Japan. *Palythoa tuberculosa* were collected from a reef at the volcanic island of Iwotorishima in southern Japan; specimens from a high pCO_2 site and from a nearby control (normal pCO_2) site (Inoue et al. in Nat Clim Change 3:683–687, 2013). We observed a statistically significant reduction in *Cladocopium* diversity at the high pCO_2 site with only one *Cladocopium* lineage present, compared to at the control site with two lineages present. Our results demonstrate that higher pCO_2 can potentially negatively influence the diversity of host Symbiodiniaceae within anthozoan hosts, an important implication in the face of ongoing ocean acidification and climate change.

Keywords Symbiodiniaceae · *Cladocopium* · Zoantharian · Acidified Reef · Iwotorishima · psbA^{ncr}

Introduction

Carbon dioxide (CO_2) from anthropogenic sources is one critical component of climate change (Orr et al. 2005; Solomon et al. 2009). The greenhouse gas is acidic by nature in its aqueous form (Orr et al. 2005; Doney et al. 2009; Solomon et al. 2009). Increased partial CO_2 pressure (pCO_2) in the atmosphere causes pCO_2 in the sea to rise via diffusion due to Henry's law of partial pressure and carbon sequestration by the ocean (Watson et al. 2009). This subsequently increases the acidity and reduces the pH of seawater, and this phenomenon is called ocean acidification (OA) (Caldeira and Wickett 2003). Furthermore, with recent ocean warming, the role of the Pacific Ocean as a CO_2 sink has been reversed and the region has become a source of CO_2 from late 2013 (Sutton et al. 2017). This situation may accelerate OA, putting additional anthropogenic pressure on marine organisms.

The increase in pCO_2 changes seawater's chemical composition and affects various biochemical cycles (Schmittner et al. 2008; Doney et al. 2009). One well-documented effect of OA is the disruption of the ocean carbonate cycle and a reduction in the saturation of calcium carbonate in the ocean (Orr et al. 2005; Kleypas et al. 2006). Thus, the reduction in pH reduces the rate of calcium carbonate skeletal formation of many organisms, including reef-forming scleractinian corals (Hoegh-Guldberg et al. 2007). In contrast, non-calcifying anthozoans

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with photosynthetic Symbiodiniaceae symbionts (such as sea anemones and zoantharians) might comparably thrive in an OA future (Inoue et al. 2013; Horwitz et al. 2015; Ventura et al. 2016). Hence, OA may drive future ecosystem shifts in favour of OA-tolerant organisms and communities (Lidbury et al. 2012; Kroeker et al. 2013).

Aside from disrupting calcium carbonate formation, OA may also affect cnidarian–Symbiodiniaceae relationships (Pecheux 2002; Anthony et al. 2008; Brading et al. 2011; Wooldridge 2012). Coral holobionts were reported to be susceptible to bleaching (loss of Symbiodiniaceae) with the rise of CO_2 concentrations in seawater (Anthony et al. 2008). Additionally, Brading et al. (2011) showed certain free-living Symbiodiniaceae phylotypes thrive under high pCO2 conditions (up to 800 ppm). These results suggest that cnidarian hosts may need to associate with certain unique or different Symbiodiniaceae species in high pCO_2 environments in order to survive (Brading et al. 2011). However, a study at a volcanic reef in Papua New Guinea examining Symbiodiniaceae in six scleractinian coral species via sequences of the nuclear internal transcribed spacer 2 (ITS2) region saw no differences in diversity between colonies at exposed (pCO_2 900 ppm) and control (pCO_2 390 ppm) sites (Noonan et al. 2013).

A recent study of an acidified coral reef at Iwotorishima, a volcanic island in southern Japan, found a shift in benthic coral community composition in regions near an underwater gas vent that raised the pCO_2 of the surrounding seawater (Inoue et al. 2013). The Iwotorishima coral reef is approximately 200 m wide from shore to reef edge, and the pCO_2 levels vary considerably from 225 to 1465 ppm (Inoue et al. 2013). Reef-building scleractinian coral cover diminishes closer to the gas vent, likely due to increasing acidity (high pCO_2 concentration) in the seawater. Only the zooxanthellate zoantharian *Palythoa tuberculosa* and the soft coral *Sarcophyton elegans* were observed to be present near the gas vent. Studies have found that *Sarcophyton* soft corals can withstand acidified environments to a certain degree ($\sim$ pH 8.01) due to their ability to mitigate stress, but their survivorship does not improve in highly acidic environments (Inoue et al. 2013; Januar et al. 2016, 2017). However, the abundance of *Sarcophyton* dropped abruptly in the region closest to the vent with extremely high pCO_2 (1465 ppm). On the other hand, compared to *Sarcophyton*, the abundance of *P. tuberculosa* was relatively not as reduced near the gas vent (Inoue et al. 2013). Similarly, other reports provide evidence of the high tolerance of *P. tuberculosa* to a wide variety of environments such as river mouths with possible lowered salinity and intertidal areas exposed to extreme temperatures (Yang et al. 2013; Reimer et al. 2017; Noda et al. 2017).

The symbionts of *P. tuberculosa* have been well studied due to the host species' wide distribution across the Indo-Pacific and its ease of identification in the field (Polak et al. 2011; Reimer 2010; Reimer et al. 2017). Burnett (2002) investigated the Symbiodiniaceae composition in *P. tuberculosa* across the Indo-Pacific Ocean, showing *Cladocopium* (former *Symbiodinium* 'Clade C', LaJeunesse et al. 2018) was the main Symbiodiniaceae group with *Durusdinium* (former *Symbiodinium* 'Clade D') present only in Southeast Asia. Subsequently, other studies have demonstrated variation in *Cladocopium* types within *P. tuberculosa* in southern Japan (Reimer et al. 2006), Taiwan (Reimer et al. 2013), Singapore (Reimer and Todd 2009), and the Red Sea (Reimer et al. 2017). More recently, with the examination of high-resolution chloroplast psbA non-coding region ($psbA^{ncr}$) sequences (LaJeunesse and Thornhill 2011), evidence of 'specialist' lineages of *Cladocopium* within *P. tuberculosa* from different marine environments has been reported (Reimer et al. 2017), including from Okinawa in southern Japan (Noda et al. 2017). In Okinawa, variation in *Cladocopium* has been observed across both smaller geographical (e.g. < 10 km) and environmental scales (e.g. depth, river mouths) than previously have been considered, and these observations have been proposed as potentially important in structuring *Cladocopium* and Symbiodiniaceae within *P. tuberculosa* (Noda et al. 2017).

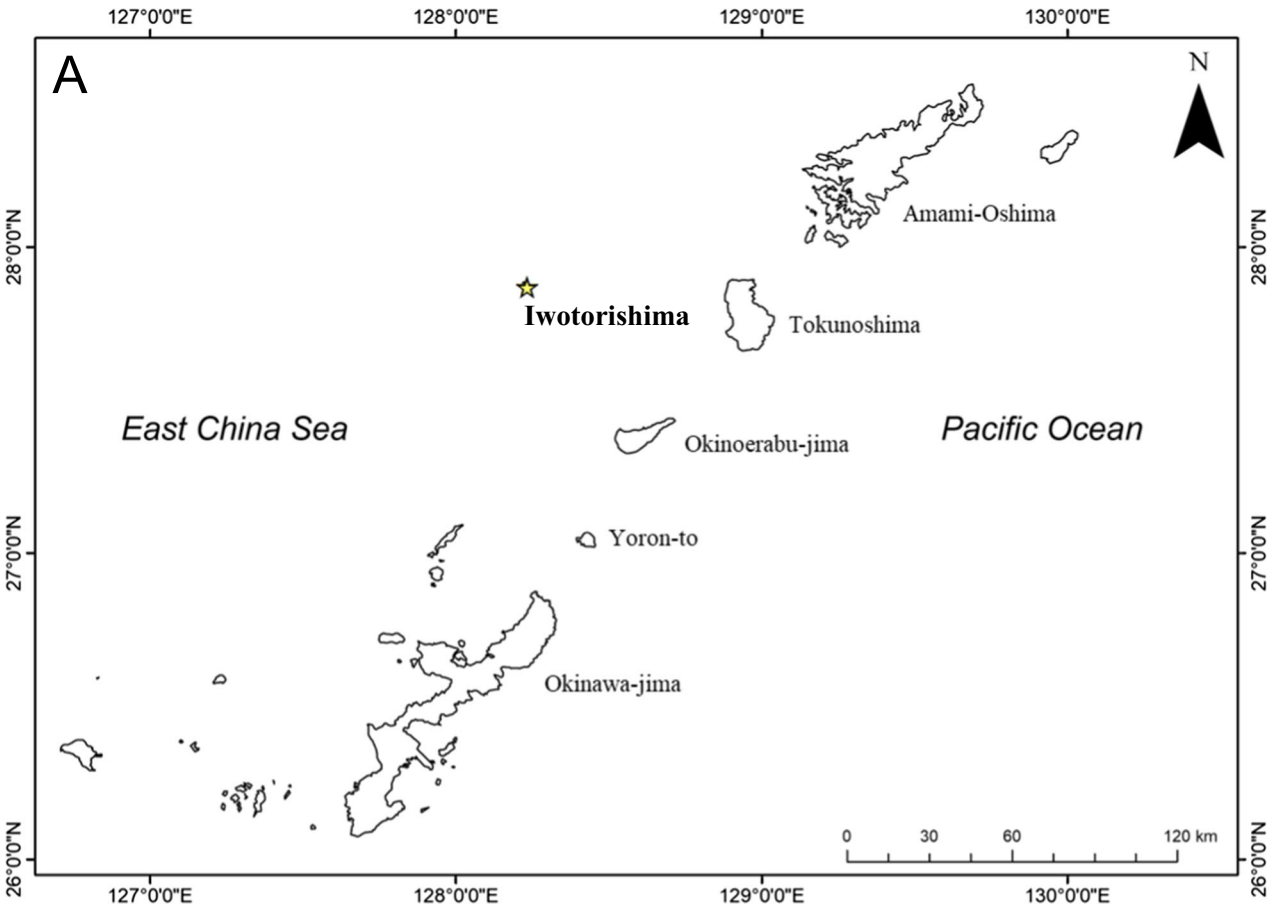
However, only limited studies have been conducted on the influence of acidified seawater to Symbiodiniaceae diversity in the field due to the rarity of such marine environments, and no studies using hypervariable $psbA^{ncr}$ sequences have been performed. The evidently high tolerance of *P. tuberculosa* to the highly acidified seawater at Iwotorishima offers the chance to investigate these issues. Therefore, in this study, we investigated the diversity of Symbiodiniaceae harboured by *P. tuberculosa* found in acidified and non-acidified locations at the Iwotorishima reef and report our findings.

Fig. 1 Location of the study site at Iwotorishima, Japan. **a** Location of Iwotorishima in relation to Okinawa-jima Island and Amami-Oshima Island. **b** Location of the bay and reef with underwater volcanic vent in white dotted line box. **c** Transect locations within the bay, showing the transect with high partial pressure of pCO_2 (red, L15) and the control site (yellow, L08) with normal pCO_2 levels

Materials and methods

Specimen collection

Iwotorishima Island is an uninhabited volcanic island 66 km west of Tokunoshima Island and 110 km north-west of Okinawa-jima Main Island (Fig. 1a). A 200 m wide, semi-enclosed coral reef is located on the southeast coast of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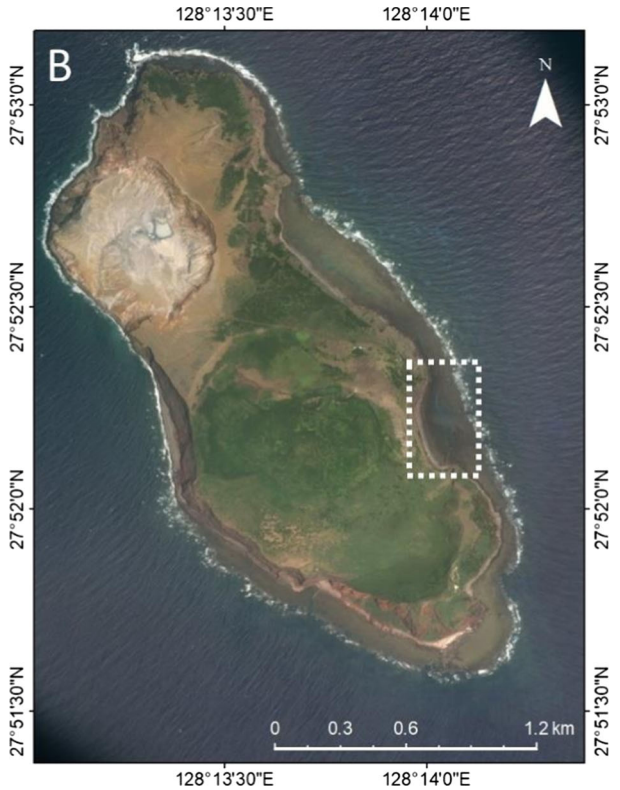
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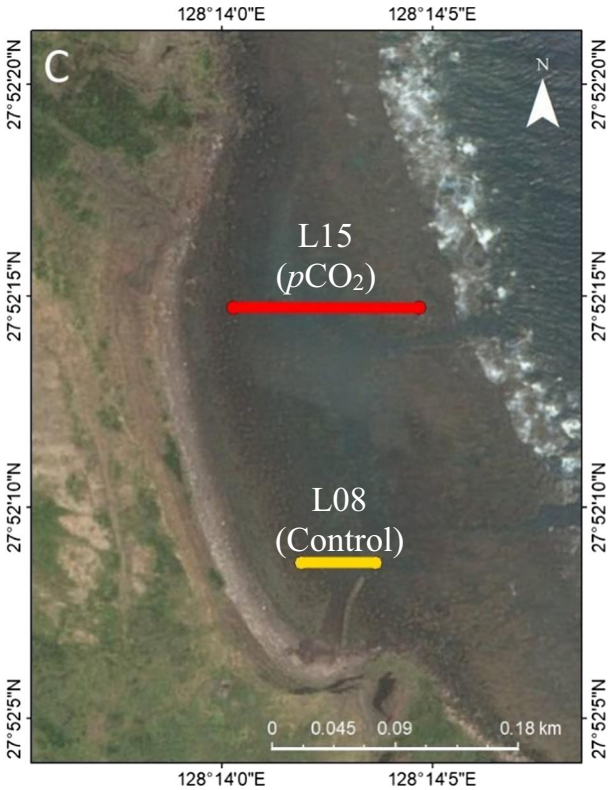
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27°52'10"N
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L15
(pCO_2)
L08
(Control)
0 0.045 0.09 0.18 km

the island with a volcanic gas vent located underwater in the northern part of the reef (Fig. 1b). On October 26–27, 2016, the reef (27°52′14.72″N, 128°14′0.29″E) was surveyed; two sites were selected based on Inoue et al. (2013), one near the underwater volcanic gas vent with high partial pressure of carbon dioxide (= transect L15 in Inoue et al. (2013), high pCO_2), and another more southern site with normal pCO_2 levels (= transect L08 in Inoue et al. (2013), control site). The sites were approximately 200 m apart from each other (Fig. 1c). L15 is within the gas bubbling area with freshwater input from a spring on the coast east of the reef.

10 specimens (colonies) of the zooxanthellate zoantharian *P. tuberculosa* were collected each from sites L15 and L08 at depths < 2 m via snorkelling. In situ photographs were taken of each colony before collecting approximately 6 cm^2 of tissue from each colony with a knife. Tissue specimens were fixed individually and stored in 99% molecular grade ethanol until further molecular analyses.

DNA extraction and PCR

Genomic DNA was extracted from specimens using a DNeasy Blood and Tissue extraction kit following the manufacturer's instructions (Qiagen, Tokyo). Two DNA marker regions were amplified via polymerase chain reaction (PCR) with two Symbiodiniaceae DNA markers targeting specific regions: the internal transcribed spacer 2 (ITS2) of nuclear ribosomal DNA and the non-coding region of the plastid minicircle ($psbA^{ncr}$). The ITS2 sequences from this study were utilized to place symbiont types within the well-established ITS2 phylogenetic framework (LaJeunesse and Thornhill 2011). On the other hand, the $psbA^{ncr}$ sequences were utilized to examine differences at a finer phylogenetic resolution (LaJeunesse and Thornhill 2011; Reimer et al. 2017; Noda et al. 2017).

The ITS2 region was amplified using the primers zITSf (5′-CCG GTG AAT TAT TCG GAC TGACGC AGT-3′) and ITS4 (5′-TCC TCC GCT TAT TGATAT GC-3′) (White et al. 1990; Rowan and Powers 1992; Hunter et al. 1997), while $psbA^{ncr}$ was amplified using the primers 7.4-Forw (5′-GCA TGA AAG AAA TGC ACA CAA CTT CCC-3′) and 7.8-Rev (5′-GGT TCT CTT ATT CCA TCA ATA TCT ACT G-3′) (LaJeunesse and Thornhill 2011; Moore et al. 2003). The PCR mixes (20 µl) were composed of 1.0 µl of genomic DNA, 10.0 µl of HotStarTaq Plus Master Mix, 1.0 µl of each primer (10 pmol), 1.0 µl $MgCl_2$ (25 mmol), 0.5 bovine serum albumin (20 mg ml^{-1}), and 1.5 µl coral load. Thermocycle conditions were modified slightly from Noda et al. (2017): ITS2: 95.0 °C for 5 min; 35 cycles of 94.0 °C for 30 s, 51.0 °C for 45 s, and 72.0 °C for 2 min; with a final extension at 72.0 °C for 10 min; and for $psbA^{ncr}$: 95.0 °C for 5 min; 40 cycles of 94.0 °C for 10 s, 55.0 °C for 30 s, and 72.0 °C for 2 min; with a final extension at 72.0 °C for 10 min. The products were sent to Fasmac (Kanagawa, Japan) for sequencing in both directions.

Phylogenetic analyses

The nucleotide sequences of ITS2 and $psbA^{ncr}$ acquired were edited using Bioedit (Hall 1999) and aligned separately within Molecular Evolutionary Genetic Analysis (MEGA) version 7 (Kumar et al. 2016). Each alignment was inspected manually, and primer regions and uneven tail ends were excluded. Because the $psbA^{ncr}$ forward and reverse reads often did not overlap (e.g. Noda et al. 2017), the reverse reads of $psbA^{ncr}$ were used for examining genotypes in this study. Other studies have utilizing $psbA^{ncr}$ have also analysed sequences in one direction (Noda et al. 2017; Reimer et al. 2017; Kunihiro and Reimer 2018). Previous reported *Cladocopium* ITS2 region sequences from *P. tuberculosa* in southern Japan were obtained from GenBank and incorporated into the ITS2 alignment as references (DQ480631, DQ480639, DQ889741, DQ889743 from *Cladocopium* subclade C1 or related) along with one sequence from *Cladocopium* subclade C15 isolated from *Zoanthus* sp. in southern Japan (AB207184). The final ITS2 alignment comprised 20 sequences of 497 bp in length.

There were no previously reported reverse $psbA^{ncr}$ sequences of *Cladocopium* extracted from *P. tuberculosa.* In order for comparison, forward sequences of $psbA^{ncr}$ with good reads ($n = 10$, 232 bp) from this study were compared with sequences from *Cladocopium* isolated from *P. tuberculosa* in Okinawa, Japan. The sequences of each lineage reported from Noda et al. (2017) were obtained from GenBank for reference: Lineage 1 (MF593447; MF593427), Lineage 2 (MF593415, MF593409), Lineage 3 (MF593405, MF493402), and Lineage 4 (MF593407; MF593406). The final reverse $psbA^{ncr}$ alignment contained 20 sequences and was 377 bp in length (Supplementary 1 and 2). Novel sequences generated by this study have been deposited in GenBank (Accession Numbers MK129435–MK129454 and MK165057–MK165086).

Both alignments of ITS2 and $psbA^{ncr}$ reverse were analysed using the maximum likelihood (ML), neighbour-joining (NJ), maximum parsimony (MP), and Bayesian inference (BI) methods. ML analyses of both data sets were analysed with MEGA using NJ tree reference under automatic model selection to find best fit substitution model. Using MEGA, ML, NJ, and MP phylogeny tree reconstructions of each alignments were generated using the Jukes-Cantor model with 1000 bootstraps phylogeny test at uniform rates among sites (Hasegawa et al. 1985).

MrBayes software (Huelsenbeck and Ronquist 2001) was used to generate the phylogeny tree of Bayes Inference using the Jukes-Cantor model and parameters set for each alignment (chain length = 5,000,000; burn-in < 2,000,000). The values of branches for each reconstructed phylogeny tree of each alignment were inserted in the ML tree. The genetic distances between and within psbA^{ncr} reverse sequences were calculated in MEGA using the maximum composite likelihood model.

Furthermore, the aligned ITS2 and psbA^{ncr} sequences were exported into fasta file format. Sequences in the alignments were then converted into numbers, and statistical analyses were conducted using GenAlEx (Genetic Analysis in Excel) version 6.5 in Microsoft Excel 2013. All 20 genotypes of each marker were used in these analyses. Each sequence in each alignment was grouped based on the location represented by population (pCO_2 and control). Polymorphic nucleotide (PN) positions with ambiguous bases ("0", 9 positions) in the ITS2 data set were removed. The data sets were transformed into a pairwise genetic distance matrix based on PN positions among individual sequences. Principal coordinate analysis (PCoA) via covariance matrix with data standardization was conducted for both pairwise data sets and a graph were generated to depict the distance among sequences. Analysis of molecular variance (AMOVA) was conducted on the PN data sets to observe genetic difference between populations.

Results

Phylogenetic analyses

Two different ITS2 genotypes of *Cladocopium* were detected (Fig. 2a) within our specimens. The first type (n = 15) was found in both the control and high pCO_2 sites, and most of these sequences (n = 14) were 100% identical with previously reported *Cladocopium* subclade C1-related sequences from *P. tuberculosa* found in southern Japan (DQ480639). In the ITS2 phylogenetic tree, these sequences were within a C1-related subclade (ML = 65%, NJ = 65%, MP = 100%, BI = 0.99). Specimen S17L08 from the control site had one insertion different from the rest of the sequences C1-related. The other genotype (n = 5) was only found in the control site and differed by one base pair from the former genotype. These sequences were also identical (100%) with other previously reported *Cladocopium* subclade C1 from *P. tuberculosa* found in southern Japan (DQ889743; DQ889741). In the phylogenetic tree, both C1/C1-related groups formed a large C1 subclade (ML = 67%, NJ = 67%, MP = 100%, BI = 0.99), which was different from *Cladocopium* subclade C3. Hence, all *Cladocopium* in this study were identified as belonging to subclade C1/C1-related group based on ITS2 results.

The psbA^{ncr} phylogeny agreed mostly with the ITS2 results (Fig. 2b). In the resulting phylogenetic tree, there were two genotype groups, with one clade with 16 specimens, from both sites (ML = 100%, NJ = 100%, MP = 100%, BI = 1.00). The remainder of the sequences (n = 4) formed another clade (ML = 100%, NJ = 100%, MP = 100%, BI = 1.00), and these specimens were found only at the control site. The between-group mean distance of both psbA^{ncr} lineages was 0.136 (± 0.022), and the within-group mean distance was much lower at 0.002 (± 0.006). When comparing forward psbA^{ncr} sequences of specimens with past records (Noda et al. 2017), a larger cluster (n = 8) grouped in Lineage 1 (ML = 100%, NJ = 100%, MP = 100%, BI = 1.00) sensu Noda et al. (MF593447; MF593427), while a lesser cluster (n = 2) grouped with Lineage 4 (ML = 100%, NJ = 100%, MP = 100%, BI = 1.00) sensu Noda et al. (MF593407; MF593406) (Fig. 2c). Thus, we inferred that with the psbA^{ncr} reverse sequences, the larger clade (n = 16) was equal to Lineage 1, and the smaller (n = 4) clade to Lineage 4.

Principle Coordinate Analysis (Supplementary 3) showed two distinct clusters of *Cladocopium* genotypes, which agreed well with both the ITS2 and psbA^{ncr} phylogenetic trees. Specimens S17L08 and S13L08 had small base-pair differences from the larger C1-related subclade (ITS2) and Lineage 1 (psbA^{ncr}) subclade, respectively, and this can be seen in the PCA results. S17L08 had one ITS2 insertion base pair compared to the other C1-related subclade, while S13L08 had 28 base-pair deletions at the end of the hypervariable psbA^{ncr} reverse sequence compared to the other Lineage 1 sequences. However, neither variant was separated into a different grouping in the PCA analyses. AMOVA results showed higher variation of *Cladocopium* genotypes within locations (ITS2 = 63%, psbA^{ncr} = 69%) than variation between locations (ITS2 = 37%, psbA^{ncr} = 31%). The PhiPT values of both ITS2 (0.370) and psbA^{ncr} (0.305) were significant ($P < 0.05$), indicating significant genetic differences between the *Cladocopium* in *P. tuberculosa* at the high pCO_2 site and at the control site.

Discussion

The high pCO_2 site is within the area with gas bubbling as observed previously (Inoue et al. 2013), with measurements showing pCO_2 up to 800 µatm when the reef is semi-enclosed during low tide. Sulphuric ion content and temperature remained constant between the high pCO_2 and control sites (Inoue et al. 2013).

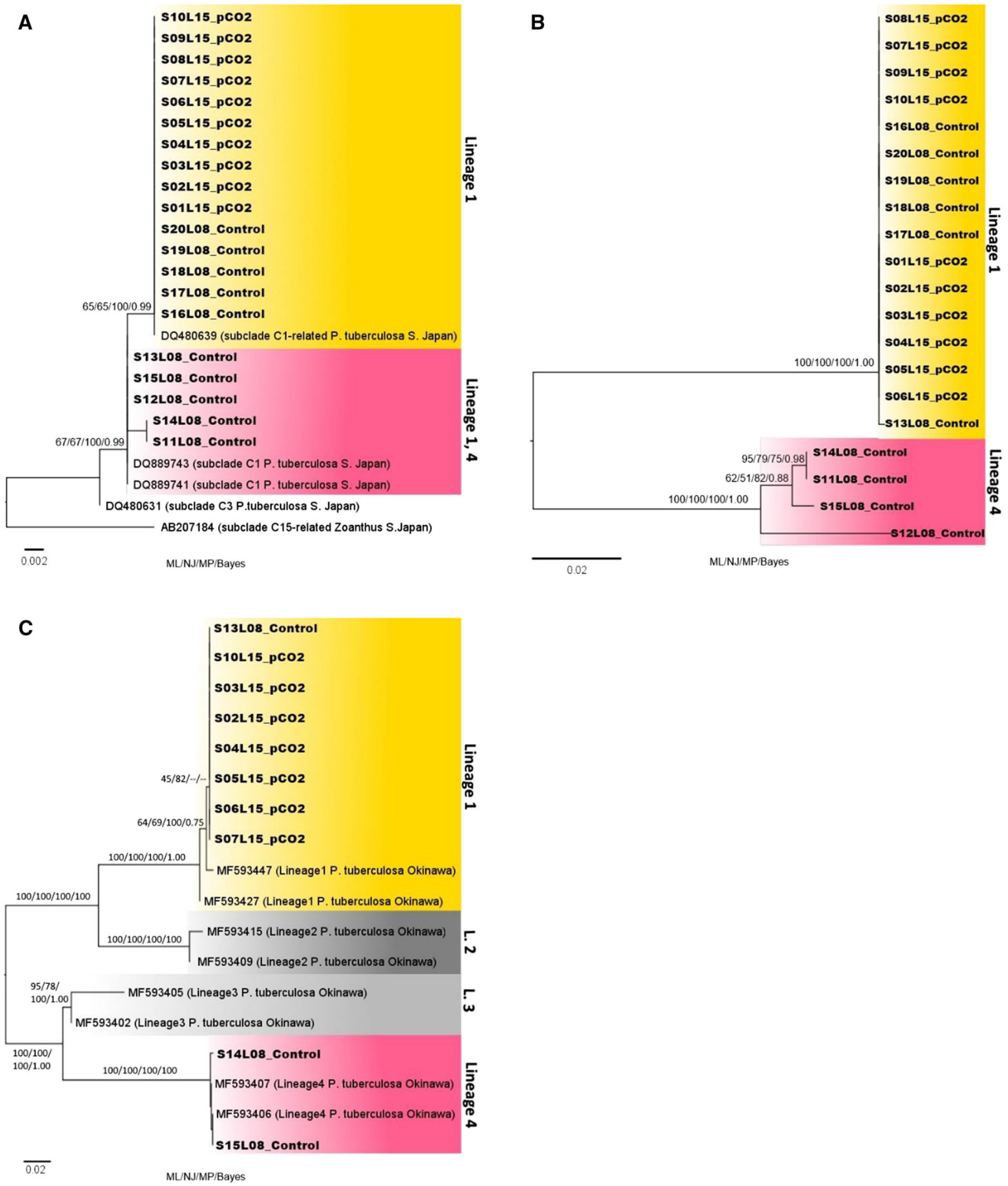

Fig. 2 Phylogenies of *Cladocopium* extracted from *Palythoa tuberculosa* found at Iwotorishima. Maximum Likelihood (ML) tree of **a** the internal transcribed spacer 2 (ITS2), and **b** reverse and **c** forward sequences of chloroplast psbA non-coding region ($psbA^{ncr}$) showing two groups/lineages consistently found in all trees. Sequences from previous studies with GenBank accession numbers, and subclades, host names, and locations are included in the ITS2 ML tree sensu *LaJeunesse* (2001). Values at the nodes represent the ML, neighbour-joining (NJ), and maximum parsimony (MP) bootstrap support, in addition to Bayesian inference (BI) posterior probabilities, respectively. Coloured boxes represent the two major genotypes of *Cladocopium* observed in this study

The phylogenetic trees and analyses of both ITS2 and psbA^{ncr} showed that there was significantly reduced *Cladocopium* genotype diversity at the high pCO_2 site. However, in our ITS2 analyses, there was only a one base-pair difference detected between the two *Cladocopium* ITS2 genotypes. This contrasts with previous studies that showed no differences in ITS2 sequences of Symbiodiniaceae in sea anemones and scleractinian corals at different pCO_2 levels (Noonan et al. 2013; Borell et al. 2014). The hypervariable psbA^{ncr} sequences generally agreed with the results from ITS2, while providing much more resolution and separation of Symbiodiniaceae genotypes (LaJeunesse and Thornhill 2011). psbA^{ncr} Lineage 1 was present at both sites and was the only observed genotype in the high pCO_2 location. This demonstrates that Lineage 1 in *P. tuberculosa* is able to survive in the acidified environment of Iwotorishima, supporting other previous research that has suggested this lineage may be a generalist (Noda et al. 2017). As for Lineage 4, their presence was restricted to only the control site. This lineage has only been recorded in low numbers ($n = 3$) in a previous study from Okinawa (Noda et al. 2017), and no conclusions have been made on its ecological nature as of yet.

Given the low numbers of specimens and sites in the current study, our results should be considered with caution. The specimens were originally collected opportunistically during a very short trip (1 d) to the island in late summer 2016. After sequencing and analyses, we realized the uniqueness of these results and attempted to collect more specimens with more control sites. However, Iwotorishima is uninhabited, far from Okinawa, and weather cancelled several planned boat trips in summers of 2017 and 2018. Regarding the lack of replication of sites, for the CO_2 seep, it is impossible to replicate as there is only one seep around Iwotorishima. Adding more specimens (and/or control sites) to this research would allow more robust conclusions to be made.

However, despite these limitations, the current results represent the first observations of a reduction of Symbiodiniaceae genotype diversity in a specific anthozoan host under increased pCO_2 conditions. This reduction in Symbiodiniaceae diversity is what would be expected under a harsh environment, with one generalist genotype dominant (Grupstra et al. 2017). Environmental parameters such as light intensity and temperature have been shown to drive both the dominance of certain genotypes of Symbiodiniaceae (Lucas et al. 2016; Silverstein et al. 2017) and the evolution of symbiodinian species (Finney et al. 2010; Tonk et al. 2013). The current results are the first indication that pCO_2 may also drive the selection of Symbiodiniaceae in hosts, as past studies have shown little to no evidence of high pCO_2 influence in the selection or dominance of Symbiodiniaceae (Noonan et al. 2013; Borell et al. 2014; Davies et al. 2018). However, these past studies only used the more conservative ITS2 region to identify Symbiodiniaceae, thus potentially missing more fine-scale differences (LaJeunesse and Thornhill 2011). The more variable psbA^{ncr} region has been shown to distinguish Symbiodiniaceae genotypes much better than ITS2, due to the hypervariable nature of this non-coding region (Reimer et al. 2017; Noda et al. 2017). However, there has only been one previous laboratory study that has utilized the psbA^{ncr} marker to identify genotypes of Symbiodiniaceae under different pCO_2 environments (Graham and Sanders 2016).

Laboratory experiments on carbon fixation rates of *Cladocopium* (subclade C1) hosted by another zoantharian, *Palythoa* aff. *clavata* (Duchassaing, 1850) under high pCO_2 conditions showed synergistic results (Graham and Sanders 2016). The *Cladocopium* C1 in Graham and Sanders (2016) is closely related to our Lineage 3, which was previously reported to have a narrow geographical distribution in the north-west part of Okinawa main island (Noda et al. 2017). In Graham and Sanders (2016), the carbon fixation of *Cladocopium* C1 decreased as pCO_2 or temperature was each independently increased. However, when tested together, the carbon fixation rate was significantly higher at high temperatures (31 °C) and extremely high pCO_2 levels (pH 7.69/$\approx$ 1037.6 ppm) (Graham and Sanders 2016). These results demonstrate that in general, zoantharians (or at least *Palythoa* spp.) may be able to thrive in high pCO_2/low pH environments as the conditions may not compromise the host–symbiont relationship.

In the field, other non-calcifying benthic cnidarians such as the sea anemone *Anemonia viridis*, found on the coast of a volcanic island in Italy, showed no changes in Symbiodiniaceae types at different pCO_2 levels (Borell et al. 2014). In order to sustain their symbiotic relationship, Symbiodiniaceae increased their physiological capability (i.e. heterotrophic rate and mitotic index), while the host regulated the density of the symbiont under high pCO_2 conditions (as high as 3232 ppm) (Towanda and Thuesen 2012; Wooldridge 2012; Horwitz et al. 2015). These previous results suggest that the host–Symbiodiniaceae relationship could be one key to the survival of non-calcifying cnidarians under elevated pCO_2 conditions.

Past studies have shown that Symbiodiniaceae are able to increase their metabolic efficiency and expand physiological capability in high pCO_2 environments, surpassing previously known limits (Wooldridge 2012; Horwitz et al. 2015; Graham and Sanders 2016). The dominance of *Cladocopium* Lineage 1 in *P. tuberculosa* close to the gas vent at Iwotorishima might be the result of this ability to adapt. While these results may confirm the generalist nature of *Cladocopium* Lineage 1 (Grupstra et al. 2017; Noda et al. 2017), it is too soon to identify this lineage as a future

dominant *Cladocopium* or Symbiodiniaceae as we know little about their physiological capability. Furthermore, there are other factors aside from Symbiodiniaceae that dictate the success of the holobiont such as geographical distribution, host specificity of symbionts, environmental tolerances of hosts, and the ability of the holobiont to adapt (Baker 2003; Tonk et al. 2013; Yang et al. 2013; Graham and Sanders 2016). Hence, in-depth studies on the host–symbiont relationship between *P. tuberculosa* and *Cladocopium* Lineage 1, especially the trophic niche interaction within the holobiont, need to be undertaken in the future to understand the physiology capability of this lineage.

Our results demonstrate that higher pCO_2 can potentially negatively influence the diversity of symbiotic Symbiodiniaceae, an important implication in the face of ongoing ocean acidification and climate change.

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Compliance with ethical standards

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

References

Anthony KRN, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. Proceedings of the National Academy of Sciences 105:17442–17446

Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: Diversity, ecology, and biogeography of *Symbiodinium*. Annual Review of Ecology, Evolution, and Systematics 34:661–689

Borell EM, Steinke M, Horwitz R, Fine M (2014) Increasing *p*CO2 correlates with low concentrations of intracellular dimethylsulfoniopropionate in the sea anemone *Anemonia viridis*. Ecology and Evolution 4:441–449

Brading P, Warner ME, Davey P, Smith DJ, Achterberg EP, Suggett DJ (2011) Differential effects of ocean acidification on growth and photosynthesis among phylotypes of *Symbiodinium* (Dinophyceae). Limnology and Oceanography 56:927–938

Burnett WJ (2002) Longitudinal variation in algal symbionts (zooxanthellae) from the Indian Ocean zoanthid Palythoa caesia. Marine Ecology Progress Series 234:105–109

Caldeira K, Wickett ME (2003) Oceanography: Anthropogenic carbon and ocean pH. Nature 425:365

Davies SW, Ries JB, Marchetti A, Castillo KD (2018) *Symbiodinium* functional diversity in the coral *Siderastrea siderea* is influenced by thermal stress and reef environment, but not ocean acidification. Frontiers in Marine Science 5:150

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO_2 problem. Annual Review of Marine Science 1:169–192

Finney JC, Pettay DT, Sampayo EM, Warner ME, Oxenford HA, LaJeunesse TC (2010) The relative significance of host-habitat, depth, and geography on the ecology, endemism, and speciation of coral endosymbionts in the genus *Symbiodinium*. Microbial Ecology 60:250–263

Graham ER, Sanders RW (2016) Species-specific photosynthetic responses of symbiotic zoanthids to thermal stress and ocean acidification. Marine Ecology 37:442–458

Grupstra CG, Coma R, Ribes M, Leydet KP, Parkinson JE, McDonald K, Catlla M, Voolstra CR, Hellberg ME, Coffroth MA (2017) Evidence for coral range expansion accompanied by reduced diversity of *Symbiodinium* genotypes. Coral Reefs 36:981–985

Hall TA (1999) BioEdit. Nucleic Acids Symposium Series 41:95–98

Hasegawa M, Kishino H, Yano TA (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160–174

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742

Horwitz R, Borell EM, Yam R, Shemesh A, Fine M (2015) Natural high pCO_2 increases autotrophy in *Anemonia viridis* (Anthozoa) as revealed from stable isotope (C, N) analysis. Scientific Reports 5:1–9

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755

Hunter CL, Morden CW, Smith CM (1997) The utility of ITS sequences in assessing the relationships among zooxanthellae and corals. Proceedings of the 8th International Coral Reef Symposium 2:99–1602

Inoue S, Kayanne H, Yamamoto S, Kurihara H (2013) Spatial community shift from hard to soft corals in acidified water. Nature Climate Change 3:683–687

Januar HI, Zamani NP, Soedarma D, Chasanah E (2016) Changes in soft coral *Sarcophyton* sp. abundance and cytotoxicity at volcanic CO_2 seeps in Indonesia. AIMS Environmental Science 3:239–248

Januar HI, Zamani NP, Soedharma D, Chasanah E (2017) Cembranoids content of soft coral *Sarcophyton* from acidified environment at volcano island, Indonesia. Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology 12:35–40

Kleypas JA, Feely RA, Fabry VJ, Langdon C, Sabine CL, Robbins LL (2006) Impacts of ocean acidification on coral reefs and other marine calcifiers: A guide for future research, report of a workshop held in 18-20 April 2005, St, Petersburg, FL, sponsored by NSF, NOAA, and the U.S. Geological Survey, 88 pp

Kroeker KJ, Micheli F, Gambi MC (2013) Ocean acidification causes ecosystem shifts via altered competitive interactions. Nature Climate Change 3:156–159

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870–1874

Kunihiro S, Reimer JD (2018) Phylogenetic analyses of *Symbiodinium* isolated from *Waminoa* and their anthozoan hosts in the Ryukyu Archipelago, southern Japan. Symbiosis: 1-12

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. Journal of Phycology 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 6:11

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. Current Biology. https://doi.org/10.1016/j.cub.2018.07.008

Lidbury I, Johnson V, Hall-Spencer JM, Mun CB, Cunliffe M (2012) Community-level response of coastal microbial biofilms to ocean acidification in a natural carbon dioxide vent ecosystem. Marine Pollution Bulletin 64:1063–1066

Lucas MQ, Stat M, Smith MC, Weil E, Schizas NV (2016) *Symbiodinium* (internal transcribed spacer 2) diversity in the coral host *Agaricia lamarcki* (Cnidaria: Scleractinia) between shallow and mesophotic reefs in the northern Caribbean (20–70 m). Marine Ecology 37:1079–1087

Moore RB, Ferguson KM, Loh WKW, Hoegh-Guldberg O, Carter DA (2003) Highly organized structure in the non-coding region of the psbA minicircle from clade C *Symbiodinium*. International Journal of Systematic and Evolutionary Microbiology 53:1725–1734

Noda H, Parkinson JE, Yang SY, Reimer JD (2017) A preliminary survey of zoantharian endosymbionts shows high genetic variation over small geographic scales on Okinawa-jima Island, Japan. PeerJ 5. https://doi.org/10.7717/peerj.3740

Noonan SHC, Fabricius KE, Humphrey C (2013) *Symbiodinium* community composition in scleractinian corals is not affected by life-long exposure to elevated carbon dioxide. PLoS ONE 8. https://doi.org/10.1371/journal.pone.0063985

Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–686

Pecheux M (2002) CO_2 Increase, a direct cause of coral reef mass bleaching. Marine Life 12:63–68

Polak O, Loya Y, Brickner I, Kramarski-Winter E, Benayahu Y (2011) The widely-distributed Indo-Pacific zoanthid *Palythoa tuberculosa*: a sexually conservative strategist. Bulletin of Marine Science 87:605–621

Reimer JD (2010) Key to field identification of shallow water brachycnemic zoanthids (Order Zoantharia: Suborder Brachycnemina) present in Okinawa. Galaxea, Journal of Coral Reef Studies 12:23–29

Reimer JD, Herrera M, Gatins R, Roberts MB, Parkinson JE, Berumen ML (2017) Latitudinal variation in the symbiotic dinoflagellate *Symbiodinium* of the common reef zoantharian *Palythoa tuberculosa* on the Saudi Arabian coast of the Red Sea. Journal of Biogeography: 1–13

Reimer JD, Irei Y, Fujii T, Yang SY (2013) Molecular analyses of shallow-water zooxanthellate zoanthids (Cnidaria: Hexacorallia) from Taiwan and their *Symbiodinium* spp. Zoological Studies 52:16

Reimer JD, Takishita K, Ono S, Maruyama T, Tsukahara J (2006) Latitudinal and intracolony ITS-rDNA sequence variation in the symbiotic dinoflagellate genus *Symbiodinium* (Dinophyceae) in *Zoanthus sansibaricus* (Anthozoa: Hexacorallia). Phycological Research 54:122–132

Reimer JD, Todd PA (2009) Preliminary molecular examination of zooxanthellate zoanthids (Hexacorallia: Zoantharia) and associated zooxanthellae (*Symbiodinium* spp.) diversity in Singapore. The Raffles Bulletin of Zoology 22:103–120

Rowan, R., & Powers, D. A. (1992). Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). *Proceedings of the National Academy of Sciences USA*, *89*, 3639–3643.

Schmittner A, Oschilies A, Matthews HD, Galbraith ED (2008) Future changes in climate, ocean circulation, ecosystems, and biogeochemical cycling for a business-as-usual CO_2 emission scenario until year 4000 AD. Global Biogeochemical Cycles 22:GB1013

Silverstein RN, Cunning R, Baker AC (2017) Tenacious D: *Symbiodinium* in clade D remain in reef corals at both high and low temperature extremes despite impairment. The Journal of Experimental Biology. https://doi.org/10.1242/jeb.148239

Solomon S, Plattner GK, Knutti R, Friedlingstein P (2009) Irreversible climate change due to carbon dioxide emissions. Proceedings of the National Academy of Sciences of the United States of America 106:1704–1709

Sutton AJ, Wanninkhof R, Sabine CL, Feely RA, Cronin MF, Weller RA (2017) Variability and trends in surface seawater pCO_2 and CO_2 flux in the Pacific Ocean. Geophysical Research Letters 44:5627–5636

Tonk L, Sampayo EM, Weeks S, Magno-Canto M, Hoegh-Guldberg O (2013) Host-specific interactions with environmental factors shape the distribution of *Symbiodinium* across the Great Barrier Reef. PLoS ONE 8:14

Towanda T, Thuesen EV (2012) Prolonged exposure to elevated CO_2 promotes growth of the algal symbiont *Symbiodinium muscatinei* in the intertidal sea anemone *Anthopleura elegantissima*. Biology Open 1:615–621

Ventura P, Jarrold MD, Merle PL, Barnay-Verdier S, Zamoum T, Rodolfo-Metalpa R, Calosi P, Furla P (2016) Resilience to ocean acidification: Decreased carbonic anhydrase activity in sea anemones under high pCO_2 conditions. Marine Ecology Progress Series 559:257–263

Watson AJ, Schuster U, Bakker DC, Bates NR, Corbière A, González-Dávila M, Friedrich T, Hauck J, Heinze C, Johannessen T, Körtzinger A (2009) Tracking the variable North Atlantic sink for atmospheric CO_2. Science 326:1391–1393

White TJ, Bruns T, Lee SJWT, Taylor JL (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18:315–322

Wooldridge SA (2012) A hypothesis linking sub-optimal seawater pCO_2 conditions for cnidarian-*Symbiodinium* symbioses with the exceedance of the interglacial threshold (> 260 ppmv). Biogeosciences 9:1709–1723

Yang SY, Bourgeois C, Ashworth C, Reimer JD (2013) *Palythoa* zoanthid 'barrens' in Okinawa: examination of possible environmental causes. Zoological Studies 52:11