Specificity trumps flexibility - location-based stable associations between Symbiodiniaceae genera and *Platygyra verweyi* (Scleractinia; Merulinidae)

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Specificity trumps flexibility—location-based stable associations between Symbiodiniaceae genera and *Platygyra verweyi* (Scleractinia; Merulinidae)

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Abstract

This study monitored symbiont communities bi-monthly in native coral cores used in a reciprocal transplantation of the coral *Platygyra verweyi* over two years (2014-2016) and samples of mother colonies from three locations with variable thermal regimes; our results show that associating with multiple Symbiodiniaceae genera (*Cladocopium* spp. and *Durusdinium* spp.) is not a prerequisite for symbiont shuffling. *Platygyra verweyi* associates with certain Symbiodiniaceae genera based on location. Results of quantitative real-time PCR indicated small-scale temporal changes in Symbiodiniaceae genera compositions from 2014-2016; however, these changes were not enough to invoke shuffling or switching, despite degree heating weeks exceeding 6°C-weeks in 2014 and 4°C-weeks in 2015, which usually resulted in substantial coral bleaching. Microsatellite analysis of the *P. verweyi* host showed no genetic differences among the study locations. Our results suggest that *P. verweyi* undergoes long-term acclimatization and/or adaptation based on microgeographic and local environmental conditions by altering its combinations of associated Symbiodiniaceae. Results also suggest that shuffling might not be as common a phenomenon as it has been given credit for; corals thrive through specific associations, and many corals could still be vulnerable to climate change-induced stress, despite being promiscuous or able to associate with rare and background Symbiodiniaceae genera.

Key Words: Reciprocal transplantation, Thermal histories, Degree heating weeks, Symbiodiniaceae, Nuclear power plant
Background

Corals in reefs around the world have been facing rapid declines in health over the past several decades due to increased and prolonged occurrences of climate change-induced seawater temperature anomalies, which often pass their threshold limits [1-3]. A common manifestation of this stress is coral bleaching due to the breakdown of coral-Symbiodiniaceae associations [4]. Moreover, corals have the potential to acclimate to climate change-induced stressors—over a short period of time (single generation)—through phenotypic plasticity or associating with specific combinations of stress resistant Symbiodiniaceae genera through natural selection; this may be the overriding determinant of their survival [5]. However, a beneficial association between a coral host and Symbiodiniaceae is a complex and holistic process that depends on whether the relationship that the coral host has with the Symbiodiniaceae genera is specific or flexible [6-8]. Corals are known to associate with a wide range of Symbiodiniaceae genera. There are nine genera of Symbiodiniaceae, and each has its own characteristic traits that help its coral host survive in a wide range of environmental niches [9]. Studies have shown that symbiosis between coral hosts and different Symbiodiniaceae genera contributes to the divergence in coral thermal tolerance under different environmental conditions [7,10]. For instance, species of Durusdinium are considered to be heat tolerant [6,11-13]; most species Cladocopium are stress sensitive but several are relatively stress tolerant (e.g. in-host Cladocopium C15, Cladocopium thermophilum) [14,15]. Durusdinium-associated corals are also known to inhabit reef environments that experience large fluctuations in surface seawater temperature [7,16,17] and be more resilient to heat-treatment experiments [18].

One widely-known mechanism that helps some corals acclimate to stressful environments is shuffling and/or switching their associated Symbiodiniaceae genera [19-25]. It has been proposed that coral hosts adjust to increasing seawater temperatures using switching—which involves existing Symbiodinium being expelled and replaced by novel Symbiodinium from the environment—and shuffling between stress-sensitive (generally Cladocopium sp.) and stress-resistant types (generally Durusdinium sp.) in the existing symbiont communities [19, 20, 26, 27]. Shuffling between Symbiodiniaceae genera has been found to benefit some coral species [11, 12] because increasing the abundance of stress-tolerant Symbiodiniaceae genera in a multi-symbiont association helps corals withstand above-threshold seawater temperature anomalies [16-18]. A transition from thermally sensitive to tolerant dominant symbionts can increase the likelihood that...
corals survive thermally-induced bleaching [28]. However, a later study [29] argued that not all corals can change their symbionts, because the mechanism of shuffling requires that a coral species hosts multiple Symbiodiniaceae genera (at least one stress tolerant and one stress resistant). Some coral species have the ability to fluctuate between Symbiodiniaceae genera on a temporal scale (e.g. [30]). The occurrence of multiple Symbiodiniaceae genera at low densities might lead to either shuffling or switching to beneficial Symbiodiniaceae genera over time [30]. In some cases, the coral host may revert back to its original composition of either a single dominant Symbiodiniaceae species or multiple species/genera (see [31]). However, there are also many cases in which the host maintains stable symbiosis with a particular Symbiodiniaceae genus, irrespective of environmental perturbations (see [31,32]). This acclimatization mechanism, although limited, may help corals survive the effects of ocean warming in the near future [20, 33].

In order to assess whether associating with multiple Symbiodiniaceae is in itself enough for shuffling to happen, we analysed coral samples collected over time from locations with different thermal regimes to determine how location influences corals’ abilities to shuffle and subsequently survive. Nanwan, Kenting National Park, Taiwan is a reef site located southwest of the third Nuclear Power Plant Outlet (NPP-OL) that has been affected by the continuous discharge of thermal effluent flowing directly into the existing coral community as a result of the near-shore current [34] since the power plant opened in 1984. NPP-OL has seawater temperatures similar to those predicted for oceans around the world by 2050 [1], making it an ideal location to conduct studies related to the effects of climate change. Nuclear power plant -OL has significantly different community compositions and settlement patterns compared to other sites such as the Nuclear Power Plant Inlet (NPP-IL) [35], suggesting that the thermal effluent has had a great impact on its benthic invertebrate and fish communities. Corals present at NPP-OL have also experienced several bleaching events over time (see [36,37]). The coral communities in the shallow water (3 m) are dominated by thermally tolerant symbiont types [30,38,39]. The increasing prevalence of stress-tolerant Durusdinium sp. at reef sites closer to NPP-OL reflects the consequences of long-term thermal effects; this prevalence also makes NPP-OL an ideal site to study holobiont dynamics under thermal stress, although other physical differences (temperature fluctuations, upwelling, and internal waves; see [40]) between NPP-OL and other sites could also be involved [30,38,39,41,42].
In this study, we collected data from *in situ* reciprocal transplant experiments (RTE) on nubbins of *P. verweyi* collected from NPP-OL, NPP-IL, and Wanlitung (WLT) in Kenting National Park (KNP) between 2014-2016 (see [43]). Using the samples from each location of origin (tagged mother colonies and native cores used in the transplant experiment), we tested whether corals in their native environments will undergo shuffling over time and associate with favourable Symbiodiniaceae genera.
Methods

Samples used in the experiment were collected with permission from the Kenting National Park headquarters (permit numbers 1040008112 and 1040002080).

Study area and coral species

All experiments and sampling were carried out at three locations: Nuclear Power Plant Outlet (NPP-OL), Nuclear Power Plant Inlet (NPP-IL), and Wanlitung (WLT) in Nanwan, southern Taiwan (Fig. 1). NPP-OL (21°55′54.4″N, 120°44′42.7″E) and NPP-IL (21°57′20.3″N, 120°45′14.2″E) are located within Nanwan in the Kenting National Park (KNP), Taiwan, and WLT (21°59′41.0″N 120°42′19.6″E) is located on the west coast of KNP, approximately 12 km from the nuclear power plant area. Due to the tidally-induced upwelling in Nanwan [41], the maximum daily seawater temperature fluctuation at NPP-OL and NPP-IL can exceed 8°C in the summer season. In this study, NPP-IL and WLT were both included to assess the potential role of thermal variability on P. verweyi facing prolonged thermal stress. The massive coral species P. verweyi generally occurs in shallow water (2-4 m) and was found to associate with the Symbiodiniaceae genera Cladocopium sp. and/or Durusdinium sp. in KNP [38].

Coral samples

Two types of samples were used in the experiment: the “mother colonies” were the original colonies (from the 2014-2016 transplantation experiment), and the “native cores” were samples cut from a mother colony and kept at the same location.

Tagged mother colonies at each location (five from NPP-IL in 2014, eight each from NPP-OL and WLT from 2014 to 2016) were sampled approximately every two months throughout the experimental period. During each sampling, a piece (2 cm diameter core) of coral was cut and fixed in 95% Ethanol for DNA extraction and qPCR analysis.

Samples were taken from the coral cores in the racks used in the transplantation experiment (see [43] for detailed explanation of the transplantation experiment). All samples were taken from the set of control racks installed at each location from the reciprocal transplant experiment, and hence referred to as “native cores”. Five native cores (one from each colony) from each rack in 2014 (at all three sites) and one piece (2 cm in diameter) from each of the 30 cores in 2015 (from the racks...
at NPP-OL and WLT) were sampled at each location. Sampling for the 2014 set was carried out in April and September of 2014 and January, March, May, July, September, and November of 2015. Sampling for the 2015 set was carried out in April, September, and November of 2015 and January and April of 2016. All samples were fixed in 95% Ethanol for DNA extraction and qPCR analysis.

**Seawater temperature**

The seawater temperature was recorded in situ at 30-minute intervals using data loggers (HOBO; Pendant™, USA) deployed underwater near the transplant racks (1-2 m) at each study site. The raw temperature data were transformed into Degree Heating Weeks (DHW) [44,45] to assess both the intensity and duration of the thermal stress for each experiment group. Although this indicator is typically used to monitor large-scale bleaching, it was also used in another study to assess the cumulative thermal stress on heat-treated corals, but daily [46]. DHW was calculated as follows. First, the weekly mean temperature for each study site was calculated from raw temperature data. Second, the maximum of the monthly mean temperatures (MMM; NPP-OL = 29.63°C; WLT = 29.28°C; NPP-IL = 28.37°C) was obtained from data loggers (HOBO; Pendant™, USA) deployed at each study location. Finally, the weekly mean temperature was subtracted from MMM to determine temperature anomalies; only temperatures at least 1.0°C above the MMM within the previous 12 weeks were considered anomalies and summed to obtain the DHW. The conceptual calculation equation is listed below:

\[
DHW_{\text{two}} = \sum \left[ (T_{\text{NPP-OL}} - \text{MMM}_{\text{WLT}}) \geq 1^\circ C \right]
\]

where \( T_{\text{NPP-OL}} \) is the weekly mean temperature at NPP-OL, \( \text{MMM}_{\text{WLT}} \) is the MMM of WLT, etc. The projections of DHW in Nanwan were obtained from [33] using a 1° × 1° resolution grid of reef cells located in southern Taiwan.

**DNA extraction**

DNA extraction was carried out using a salting-out method modified from [47]. Coral tissue was lysed overnight in a 2-mL Eppendorf tube with 200 µL of lysis buffer [0.25 M Tris, 0.05 M EDTA at pH 8.0, 2% sodium dodecylsulfate (SDS) and 0.1 M NaCl] and 10 µL of 10 mg/mL proteinase E at 55°C in a water bath. NaCl (210 µL at 7 M) was added to the lysed tissue in the tube, and the
sample was mixed by carefully inverting the tube. The solution was then transferred to a 2-mL collection tube containing a DNA spin column (Viogene, USA) and centrifuged at 8000 rpm for 1 min. The lysate was washed twice with 500 µL of ethanol (70%) by centrifuging at 8000 rpm for 1 min at each step, with an additional centrifugation step at 8000 rpm for 3 min to dry the spin column. The column was dried further at 37°C for 15 min, then the DNA was eluted with 50 µL of preheated (65°C) 1X TE buffer, with a final centrifugation at 15000 g for 3 min. The quality of genomic DNA was checked using a 1% agarose gel. The concentrations of genomic DNA were determined using NanoDrop 2000 (Thermal Scientific, USA).

**Real-time quantitative PCR**

The copy numbers of *Cladocopium* sp. and *Durusdinium* sp. in *P. verweyi* samples were determined under a LightCycler® 480 Instrument II (Roche, Switzerland) using a protocol modified from [48]. Each 10 µL qPCR reaction consisted of 5 µL 1x SYBR Fast Master Mix, 0.5 µL UF primer (2 nM/µL), 0.5µL CR or DR primer (2 nM/µL), 7.5 µL ddH₂O, and 2.5 µL DNA templates (equal to 1 ng of genomic DNA). The following primer sets were used: ITS1 clade C-specific reverse primer (CR) 5-AAGCATCCCTCACAGCCAAA-3, clade D-specific reverse primer (DR) 5-CACCGTAGTGGTTCACGTGTAATAG-3, and universal forward primer (UF) 5-AAGGAGAAGTCGTAACAAGGTTTCC-3 [12]. Each sample was run in triplicate (technical replicates), as was a no-template control (NTC) with ddH₂O. Plasmid standard curves were run in duplicate with *P. verweyi* samples to quantify the copy numbers of each symbiont. Plasmid standard curves were generated using PCR products from *Cladocopium* sp. and *Durusdinium* sp., which were ligated into pGem®-T Easy vectors (Promega, USA), transformed, and amplified using *E. coli*. Copy numbers of final products were calculated by first quantifying the concentration of plasmid DNA through NanoDrop 2000 (Thermal Scientific, USA), then dividing by the mass of the plasmid \[\text{mass of each plasmid copy} = 3015 \text{ bp (vector)} + 100 \text{ bp (inserted PCR product)} \times 1.096 \times 10^{-21} \text{ g/bp} = 3.4 \times 10^{-18} \text{ g}\], and finally multiplying by the plasmid DNA template volume (2.5 µL) in each reaction. Serial dilutions of 1:10 from 3x10⁶ and 30 copies of the plasmid standard containing *Cladocopium* sp. and *Durusdinium* sp. sequences, respectively, were generated in the end. The qPCR cycling settings were: 40 two-step cycles of 15 s at 95°C and 1 min at 60°C. Melting curves were generated by starting at 60°C and increasing the temperature with a ramp speed of 0.11°C/s until it reached 95°C. Fluorescence data were collected after each annealing step, and five
readings were collected every second during the melting curve analysis. Crossing points (Cp) were determined by Light Cycler 480 software version 1.5 (Roche, Switzerland) using the second derivative method, which represents the cycles with the maximum number of fluorescence signals in each sample [49]. Samples with Cp values that varied from the other two technical replicates by 1 were excluded from analysis. Samples were re-run if all Cp values of technical replicates varied from one another by 1. Since high variation occurred in Cp values (varied more than 1) within technical replicates of each sample when Cp>34, the cut-off cycle was set to 34 to avoid false positives caused by the formation of non-specific fluorescence. Average copy numbers of symbiont clades C and D were obtained individually, and the formula for determining relative symbiont abundance in this study is listed below, following a correction suggested by [48]:

\[
\frac{\text{Clade D copy numbers} / 3}{\left(\frac{\text{Clade D copy numbers}}{3}\right) + \text{Clade C copy numbers}}
\]

**Population genetics analysis of P. verweyi**

To demonstrate the presence of a genetic structure in *P. verweyi* at NPP-OL and WLT, eight polymorphic microsatellite loci were used to examine 30 *P. verweyi* colonies (including transplanted colonies) found within two transplanted sites. Three published microsatellite tetramer markers developed from *P. sinensis* [50] and *P. daedalea* [51] were used. Another five microsatellite dimer markers specific to *P. verweyi* were developed by Next Generation Sequencing approaches [52]. Eight microsatellite markers were amplified following the effective universal fluorescent labeling method [53]. Amplifications performed using 25 μL reactions contained 10 ng of DNA template, 1X of VeraSeq Buffer II (Qiagen Beverly, USA), 0.5U of VeraSeq 2.0 high-fidelity DNA polymerase (Qiagen Beverly, USA), 0.2 mM of dNTP mix, 0.08 μM of specific forward primer-attached M13 (-21) tail (5’- TGT AAA ACG ACG GCC AGT -3’ (18 bp), 0.2 μM of specific reverse primer, and 0.2 μM TAMRA-labelled universal M13 (-21) primer (5’- TGT AAA ACG ACG GCC AGT -3’) [53]. The PCR conditions were: 1 cycle at 98°C for 30 s; 25 cycles of 98°C for 10 s, specific primer annealing temperature (Table 1) for 30 s, and 72°C for 30 s; followed by 10 cycles of fluorescent-labelled M13 amplification: 98°C for 10 s, 53°C for 30 s, 72°C for 30 s; and a final elongation of 10 min at 72°C. For microsatellite genotyping, samples were electrophoresed on 5% urea denaturing polyacrylamide gels using the Gel-Scan 3000™ real-time DNA fragment analysis gel Electrophoresis System (Corbett Robotics, Australia). Allele size was detected by the software Gene Profiler 4.05 (Scanalytics) with the
internal lane size standard (GeneScan™-350 TAMRA™, Applied Biosystems). Characteristics of microsatellite loci—such as number of alleles and mean observed and expected heterozygosities—were calculated using GenAlEx v.6.502 [54]. Genepop was used on the web to test for linkage disequilibrium and significant departure from Hardy-Weinberg equilibrium (HWE). None of the loci showed HWE deviation or linkage disequilibrium after Bonferroni correction [55]. Population differentiation was inferred using ARLEQUIN v3.5 [56]. Inference population genetic structure was estimated using a Bayesian clustering approach implemented in STRUCTURE v.2.3.4 [57]. The admixture model and allele frequency correlation were used. Values of number of genetic clusters (K) from 1 to 2 were tested by running three replicate simulations per K with 1,000,000 Markov chain Monte Carlo repetitions and 100,000 burn-in iterations.

Statistical analysis

All statistical analyses in this study were performed in R version 3.1.1 [58]. Differences in daily mean seawater temperatures and daily seawater temperature fluctuations between sites were tested using Kruskal-Wallis test followed by Dunn’s post hoc test with Bonferroni adjusted p-values.
Results

Seawater temperature

Weekly average seawater temperatures were plotted from the data collected by data loggers from 2014 and 2106. Both monthly and daily average seawater temperatures at NPP-OL were significantly higher (2.0–3.0°C) than at adjacent locations (Fig. 2A). In 2014, the average summer (June to August) daily seawater temperature at NPP-OL (30.11 ± 1.07 SD°C) was different from those at WLT (29.59 ± 0.67 SD °C; Dunn’s post hoc test, p<0.001) and NPP-IL (28.61 ± 0.96 SD °C; p<0.001). In 2015, the average summer daily seawater temperature at NPP-OL (29.77 ± 1.12 SD °C) was different from that at WLT (29.52 ± 0.52 SD °C; Wilcoxon rank sum test, W=5097, p<0.05). The daily seawater temperature fluctuation at NPP-OL (2.38 ± 1.04 SD °C) was different from that at WLT (1.57 ± 0.70 SD°C) (Wilcoxon rank sum test, W=655557, p<0.001). The heating event (≥30°C) at NPP-OL occurred for a longer time each day than at WLT.

The daily seawater temperature fluctuation at NPP-OL (2.23 ± 1.00 SD °C) was different from those at WLT (1.53 ± 0.58 SD °C; p<0.001) and NPP-IL (1.80 ± 1.30 SD °C; p<0.001), while the fluctuations did not vary between WLT and NPP-IL (p=1.000). During the summer, however, the daily seawater temperature fluctuation at both NPP-OL and NPP-IL was more than 7°C (maximum 9.12°C at NPP-OL and 7.19°C at NPP-IL). The daily heating event (≥30°C) occurred for the longest time at NPP-OL.

In 2014, repeated seawater temperature anomalies (the weekly mean seawater temperature exceeding the bleaching threshold) occurring at NPP-OL during the summer, resulting in a DHW of 6.4°C-weeks, while those at NPP-IL and WLT were 2.4 and 1.0 °C-weeks respectively (Fig 2B). DHW greater than 4.0°C-weeks results in a NOAA Alert Level 1, meaning that bleaching is likely. The DHWs started to decrease gradually in the fall. In 2015, the DHWs for both NPP-OL and WLT were below the threshold limit of 4.0°C-weeks—3.8 and 1.0 for NPP-OL and WLT, respectively.

Temporal variation in Symbiodiniaceae genera associated with P. verweyi at NPP-OL
The results of the real-time qPCR analysis of the samples from the 2014 experiment (Fig. 3A) indicated that, until January 2015, the symbiont communities in the native cores of NPP-OL were dominated by *Durusdinium* spp. In March 2015, the relative proportions of symbionts changed: *Cladocopium* spp. were present at various percentages (range: 1-21%; mean: 6%; Fig. 3A), and *Durusdinium* spp. accounted for the rest. Symbiont dynamics in the core with 21% *Cladocopium* spp. did continue to fluctuate (13% in May; 18% in July and September; Fig. 4). However, this core was dead by November 2015. All remaining cores survived, fluctuating between 1-5% (mean: 2%) *Cladocopium* spp. for the entire experiment.

In the 2015 experiment (Fig. 3B), the symbiont community in the native core samples from NPP-OL were dominated by *Durusdinium* spp. However, qPCR suggested that the cores in the samples from April 2016 were associated with *Cladocopium* spp. in addition to the already present *Durusdinium* spp. Previously undetected levels of *Cladocopium* spp. increased enough to be detected by qPCR. Cores were found to contain 3-61% (mean: 22.6%) *Cladocopium* spp. in April 2016.

Bi-monthly sampling of the mother colonies revealed that *P. verweyi* was specific with respect to which Symbiodiniaceae genera it associated with (Fig. 4). Colonies sampled from NPP-OL showed changes in associated Symbiodiniaceae, meaning that none of the 30 colonies analysed were associated with 100% *Durusdinium* spp. throughout the entire study period. However, the fluctuation between *Durusdinium* spp. and *Cladocopium* spp. was not large. Colonies were found to host 91-100% *Durusdinium* spp. (seven of the nine colonies)—one colony hosted 50 and 80% at two sampling times—with the exception of one colony, which associated with 72 and 99% *Durusdinium* spp. in November 2014 and January 2015, respectively. However, this colony changed its relative proportion of symbionts to 100% *Cladocopium* spp. in March 2015, and then reverted to 97% *Durusdinium* spp. in May 2015. This was the only colony that had a large fluctuation in associated Symbiodiniaceae between the two Symbiodiniaceae genera.

Temporal variation in Symbiodiniaceae genera associated with *P. verweyi* at WLT
In the 2014 experiment samples from WLT, a predominance of *Cladocopium* spp. was observed in the majority of the cores (Fig. 3A), with the exceptions of two cores found dead from March 2015 onwards and one colony that reverted to *Durusdinium* spp. (83%).

In 2015, cores from WLT were mainly associated with *Cladocopium* spp., ranging from 5-99% (mean: 31%) (Fig. 3B). Five of the 30 cores were associated with *Durusdinium* spp. throughout the experimental period. However, in November 2015, 12 of the 30 cores were associated with *Durusdinium* spp. (range: 1-87%; mean: 32%). Three cores at WLT that were mainly associated with *Cladocopium* spp. were dead by the end of the sampling period in April 2016 (Fig. 3B).

In the case of the WLT bimonthly analysis of the samples from the mother colonies, seven of the eight colonies analysed were associated only with *Cladocopium* spp., except for one colony, which also associated with *Durusdinium* spp. (2% in November, 2014; 1% in July, 2015; and 2% in April, 2016) in addition to *Cladocopium* spp. (Fig. 4).

Temporal variation in Symbiodiniaceae genera associated with *P. verweyi* at NPP-IL

In 2014, all the cores at NPP-IL were found to be predominantly associated with *Cladocopium* spp. throughout the experimental period. None of the cores were associated with *Durusdinium* spp. (Fig. 3A).

With respect to the bimonthly analysis of the samples collected from the mother colonies, again all the samples were predominantly associated with *Cladocopium* spp. throughout the sampling period. However, some colonies did show the presence of *Durusdinium* spp. (1% in March, May, and November; 29% in October, 2015; Fig. 4).

Microsatellite analysis of host samples from NPP-OL and WLT

Microsatellite analysis revealed that a total 60 *P. verweyi* colonies (30 each from NPP-OL and WLT) exhibited six to 16 alleles per locus for all eight microsatellite loci, with a mean expected heterozygosity of 0.756 ± 0.122 (Table 1). Average gene diversity of *P. verweyi* at NPP-OL across eight loci was 0.608 ± 0.393; the average for populations at WLT was 0.657 ± 0.460. The pairwise genetic differentiation $F_{st}$ value between the two sites was -0.00814, and the p-value showed no
significant difference. The genetic structure analysis by Bayesian clustering between NPP-OL and WLT also showed no significant differences and no genetic isolation between the two locations (Fig. 5).
The present study shows spatial variation but specificity in the dominant Symbiodiniaceae genera in the coral *P. verweyi*, and this may be related to local thermal histories. What was seen in *P. verweyi* is an almost stable association with a dominant Symbiodiniaceae genus at each sampling time from 2014 to 2016. A fluctuation was observed between Symbiodiniaceae in native cores and/or mother colonies, but this was not a general phenomenon. There was a certain level of temporal and spatial fluctuation in the native coral cores and tagged mother colonies. For example, in the 2014 experiment, some cores at NPP-OL (nubbins here are associated with *Durusdinium* spp.) did acquire low percentages of *Cladocopium* spp. (in March 2015) relative to the already present *Durusdinium* spp. (Fig. 3A). A similar fluctuation was seen in one core from WLT, which was found to have acquired up to 83% *Durusdinium* spp. However, none of the cores at NPP-IL showed any fluctuation (Fig. 3A). A similar pattern was observed in the tagged mother colonies, with low levels of fluctuation and Symbiodiniaceae genera acquisition (Fig. 4).

Considering the differences in seawater temperature regimes among the three locations (Fig. 2A), this study assumed that native cores and mother colonies would show different levels of Symbiodiniaceae genera shuffling through time. However, the results showed that one Symbiodiniaceae genus was always dominant in each sample (Fig. 3, 4). For example, samples at NPP-OL were always dominated by *Durusdinium* spp. Such preference could be because corals at NPP-OL are exposed to long-term seawater temperature stress at shallow depths (1-5 m) and hence are naturally acclimatized to an association with *Durusdinium* spp.

Association with a particular dominant symbiont could help *P. verweyi* dominate shallows at various locations in KNP. During the experiment and sampling periods (2014-2016), there was one major bleaching event (2014) and several typhoons (2015). Irrespective of the type of symbiont *P. verweyi* associated with, none of the corals experienced any clear bleaching—although some degree of paling of the tissue was observed. Increased seawater temperatures in 2014 resulted in DHWs of 6, 2.5, and 1°C-weeks at NPP-OL, WLT, and NPP-IL, respectively [43]. DHW in 2015, however, was below 4°C-weeks in both NPP-OL and WLT (Fig. 2B). The lower DHW values in 2015 could be attributed to the typhoons that occurred that year: the southern coast of Taiwan was hit by three typhoons in the summer, July – September 2015, resulting in the seawater temperature cooling. Such intense changes in seawater temperature conditions might have resulted in corresponding fluctuations in associated Symbiodiniaceae genera in some cores.
or tagged mother colonies (Fig. 3, 4). Temperature anomalies found in 2014, with high DHWs, were not enough to yield any pronounced shuffling or switching in this coral. Our observations beg the following questions: how common is shuffling in corals that can associate with two different symbionts, and does flexibility in symbiont associations via shuffling aid corals under stress? Studies have pointed out that corals often associate with two or more symbionts, with one being dominant and others present at low proportions (<5%, background symbiont) [8]. Therefore, corals have potential to shuffle by regulating their proportions of background symbionts when faced with unfavourable conditions. In this study, we detected symbionts at abundances as low <1%; however, such low concentrations might not be a prerequisite for shuffling or even switching. Also, the recent use of NGS amplicon sequencing has uncovered a rare biosphere with the potential to shuffle and/or switch between different Symbiodiniaceae genera. For example, [31] investigated the Symbiodiniaceae rare biosphere in two Pocilloporid species from Lord Howe Island in the Great Barrier Reef over two years. Their results showed that, following two consecutive bleaching events, the species shuffled and became associated with new Symbiodiniaceae genera (most <1% of the relative abundance, with one resistant type reaching 33% of the relative abundance).

On the other hand, [59] suggested that a pre-stress Symbiodiniaceae (D:C) ratio of <0.003 limits the ability of corals to survive bleaching after shuffling. And another study [60] showed that variation in the presence and abundances of background or low percentages of symbionts in corals is not necessarily related to shuffling, and may have little or no importance in coral physiology. Future studies need to examine the physiological role of those rare biospheres in terms of supporting corals’ responses to stress.

It may be argued that specificity to a particular Symbiodiniaceae genus depends on the host. We performed genetic analyses on the host using mitochondrial and nuclear markers in a previous study [38] and microsatellite markers in this study. The results showed no genetic difference in the host between locations. This suggests that other factors influence the type of association we see in this coral, including possible local and microgeographic adaptations to seawater temperature. It may be that using more advanced techniques would help uncover prevalent genetic differences between hosts from two locations in KNP, as was shown in a recent study—[61], which showed a clear genetic difference in P. daedalea-associated Symbiodiniaceae between Oman and Abu Dhabi in the Persian Gulf, and hence demonstrated a difference in their eco-physiological behaviour.
Both macro- and micro-environmental differences between locations could dictate Symbiodiniaceae genera associations in *P. verweyi*. For example, typhoons and upwelling or fluctuating temperatures in shallow reef areas could raise the thermal tolerance of coral, as could the influence of fluctuating environmental factors such as tidal exposure [62]. Although we demonstrated that Symbiodiniaceae mediate *P. verweyi* acclimatization, we cannot rule out the possibility that mutations to the host itself and natural selection lie behind this species’ ability to adapt to a particular condition. The effect of micro-environment might also explain the difference seen in the Symbiodiniaceae genera association between the native cores and mother colonies. Specificity towards a particular Symbiodiniaceae genus was more apparent in the mother colonies (Fig. 4). Native cores, due to their small size and hence propensity toward stress, showed more flexibility in their associated Symbiodiniaceae genera (Fig. 3 A, B) [also see the results in 43]. We hypothesize that combinations of *P. verweyi* and Symbiodiniaceae genera tend to be specific due to the differences in thermal histories, temperature variations, and hosts favouring one dominant symbiont rather than shuffling. This could have a negative impact on coral exposed to above-threshold thermal anomalies. For example, [43] observed that, when *P. verweyi* nubbins were reciprocally transplanted between NPP-OL and WLT, those from WLT that were associated with *Cladocopium* spp. did not tolerate long-term changes in temperature levels or daily fluctuations. The transplanted nubbins did not survive, even after shuffling. In contrast, nubbins at NPP-OL survived and actually fared well in the more stable and lower-temperature environment of WLT. In fact, they also showed an increase in growth over time, all the while associating with *Durusdinium* spp., and did not shuffle to *Cladocopium* spp. even though they could have. Results from this study and symbiont association data and host population genetics at a micro-geographic scale (see [38]) hint towards local adaptation in *P. verweyi*.

Conclusions

Shuffling is not a simple and straightforward way for corals to cope with the effects of climate change, but is in fact a complex process governed by host-symbiont specificity as well as local macro- and micro-environmental conditions. Being flexible [8] is a good strategy, but specificity is also a norm. In other words, a mere increase in temperature above the threshold limit is not enough to invoke shuffling, even if a coral host has the capacity to associate with multiple symbiont partners (see [43]). While it is popular to be optimistic that shuffling or switching
between Symbiodiniaceae genera is a way for corals to survive frequent above-threshold seawater anomalies, we should be cautious, as not all coral species appear to be able to shuffle or switch their associated Symbiodiniaceae genera (e.g. [63]), especially corals that have obligate relationships with a particular Symbiodiniaceae genus. We want to reiterate here that, irrespective of corals’ temperature tolerance thresholds in the future, given the fact that we are facing continuous changes in the global climate through carbon emissions, symbiont shuffling might not be sufficient to withstand frequent and prolonged seawater temperature anomalies as it is not a common trait in all coral species.

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References

1. IPCC. 2018 Summary for Policymakers. In: Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty [V. Masson-Delmotte, P. Zhai, H. O. Pörtner, D. Roberts, J. Skea, P. R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. PIDcock, S. Connors, J. B. R. Matthews, Y. Chen, X. Zhou, M. I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, T. Waterfield (eds.)]. In Press.

2. Hughes, T. P., Kerry, J. T., Alvarez_Noriega, A., Alvarez-Romero, J. G., Anderson, K.D., Baird, A. H., Babcock, R. C., Beger, M., Bellwood, D. R., Berkelmans, R., Bridge, T. C., Butler, I. R., Byrne, M., Cantin, N. E., Comeau, S., Conolly, S. R., Cumming, G. S., Dalton, S. J., Diaz-Pulido, G., Eakin, M. C., Figueira, W. F., Gilmour, J. P., Harrison, H. B., Heron, S. F., Hoey, A. S., Hobbs, J-P. A., Hoogenboom, M. O., Kennedy, E. V., Kuo, C-Y., Lough, J. M., Lowe, R. J., Liu, G., Mc Culloch, M. T., Malcolm, H. A., McWilliam, M. J., Pandolfi, J. M., Pears, R. J., Pratchett, M. S., Schoepf, V., Simpson, T., Skirving, W. J., Sommer, B., Torda, G., Wachenfeld, D. R., Willis, B. L., Wilson, S. K. 2017 Global warming and recurrent mass bleaching of corals. NATURE 543, 373–377.

3. Hughes, T. P., Anderson, K. D., Conolly, S. R., Heron, S. F., Kerry, J. T., Mough, J. M., Baird, A. H., Baum, J. K., Beruemn, M. L., Bridge, T. C., Claar, D. C., Eakin, M. C., Gilmour, J. P., Graham, N. A. J., Harrison, H., Hobbs J-P. A., Hoey, A. S., Hoogenboom, M., Lowe, R. J., Mc Culloch, M. T., Pandolfi, J. M., Pratchett, M., Schoepf, V., Torda, G., Wilson, S. K. 2018 Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science 359, 80–83.

4. Brown, B. E. 1997 Coral bleaching: causes and consequences. Coral Reefs 16, S129–S138.

5. Marshall, P. A., Baird, A. H. 2000 Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs. 19, 155-163.

6. Baker, A. C. 2003 Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of Symbiodinium. Annual Review of Ecology, Evolution, and Systematics, 661–689.

7. Lajeunesse, T. C., Pettay, D. T., Sampayo, E. E., Phongsuwan, N., Brown, B. E., Obura, D. O., Hoegh-Guldberg, O. & Fitt, W. K. 2010 Long-standing environmental conditions, geographic isolation and host-symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus Symbiodinium. Journal of Biogeography 37, 785–800.

8. Silverstein, R. N., Correa, A. M. S. & Baker, A. C. 2012 Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change. Proceedings of the Royal Society B: Biological Sciences 279, 2609–2618.

9. Lajeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R. & Santos, S. R. 2018 Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. Current Biology. (doi:10.1016/j.cub.2018.07.008)

10. Weber, M. & Medina, M. 2012 The role of microalgal symbionts (Symbiodinium) in holobiont physiology. Advances in Botanical Research 64, 119–140.

11. Jones, A. M., Berkelmans, R., Van Oppen, M. J. H., Mieog, J. C. & Sinclair, W. 2008 A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proceedings of the Royal Society B: Biological Sciences 275, 1359–1365.

12. Sampayo, E. E., Ridgway, T., Bongaerts, P. & Hoegh-Guldberg, O. 2008 Bleaching
susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. Proceedings of the National Academy of Sciences 105, 10444–10449.

13. Ulstrup, K. E. & Van Oppen, M. 2003 Geographic and habitat partitioning of genetically distinct zooxanthellae (Symbiodinium) in Acropora corals on the Great Barrier Reef. Molecular Ecology 12, 3477–3484.

14. Fisher, P.L., Malme, M.K., Dove, S. 2012 The effect of temperature stress on coral–Symbiodinium associations containing distinct symbiont types. Coral Reefs 31, 473–485.

15. Hume, B. C. C., D’Angelo, C., Smith, E. G., Stevens, J. R., Burt, J., Wiedenmann, J 2015 Symbiodinium thermophilum sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world’s hottest sea, the Persian/Arabian Gulf. Scientific Reports 5, 8562 doi:10.1038/srep08562

16. Lien, Y.-T., Nakano, Y., Plathong, S., Fukami, H., Wang, J.-T. & Chen, C. A. C. 2007 Occurrence of the putatively heat-tolerant Symbiodinium phylotype D in high-latitude outlying coral communities. Coral Reefs 26, 35–44.

17. Ghavam Mostafavi, P., Fatemi, S. M. R., Shahhosseiny, M. H., Hoegh-Guldberg, O. & Loh, W. K. W. 2007 Predominance of clade D Symbiodinium in shallow-water reef-building corals off Kish and Larak Islands (Persian Gulf, Iran). Marine Biology 153, 25–34.

18. Oliver, T. A. & Palumbi, S. R. 2011 Many corals host thermally resistant symbionts in high-temperature habitat. Coral Reefs 30, 241–250.

19. Baker, A. C., Starger, C. J., McClanahan, T. R. & Glynn, P. W. 2004 Coral reefs: corals’ adaptive response to climate change. NATURE 430, 741.

20. Berkelmans, R. & Van Oppen, M. 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.

21. Sampayo, E. E., Ridgway, T., Bongaerts, P. & Hoegh-Guldberg, O. 2008 Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. Proceedings of the National Academy of Sciences 105, 10444–10449.

22. Jones, A. M. & Berkelmans, R. 2008 A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proceedings of the Royal Society B: Biological Sciences 275, 1359–1365.

23. Silverstein, R. N., Cunning, R. & Baker, A. C. 2015 Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. Global Change Biology 21, 236–249.

24. Cunning, R., Silverstein, R. N. & Baker, A. C. 2015 Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. Proceedings of the Royal Society B: Biological Sciences 282, 20141725–20141725.

25. Boulotte, N. M., Dalton, S. J., Carroll, A. G., Harrison, P. L., Putnam, H. M., Peplow, L. M. & van Oppen, M. J. 2016 Exploring the Symbiodinium rare biosphere provides evidence for symbiont switching in reef-building corals. The ISME journal (doi:10.1038/ismej.2016.54)

26. Buddemeier, R. W., Fautin, D. G., Ware, J. R. 1997 Acclimation, adaptation and algal symbiosis in reef-building scleractinian corals. In: Coelenterate biology: proceedings of the sixth international congress of coelenterate biology. Leiden: Nationaal Natuurhistorisch Museum. 3

27. Baker, A. 2001 Ecosystems: reef corals bleach to survive change. Nature 411, 765-766

28. Bay, L.K., Doyle, J., Logan, M., Berkelmans. R. 2016 Recovery from bleaching is mediated by
threshold densities of background thermo-tolerant symbiont types in a reef-building coral. Royal Society open science.3(6):160322. https://doi.org/10.1098/rsos.29.

Goulet T. 2006 Most corals may not change their symbionts. Marine Ecology Progress Series 321, 1-7

Hsu, C. M., Keshavmurthy, S., Denis, V. & Kuo, C. Y. 2012 Temporal and Spatial Variations in Symbiont Communities of Catch Bowl Coral Isopora palifera (Scleractinia: Acroporidae) on Reefs in Kenting National Park, Taiwan. Zoological Studies 51, 1343-1353.

Thornhill, D. J., Lajeunesse, T. C., Kemp, D. W., Fitt, W. K. & Schmidt, G. W. 2006 Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. Marine Biology 148, 711–722.

Thornhill, D. J., Fitt, W. K. & Schmidt, G. W. 2006 Highly stable symbioses among western Atlantic brooding corals. Coral Reefs 25, 515–519.

Palumbi, S. R., Barshis, D. J., Traylor-Knowles, N., Bay, R. A. 2014 Mechanisms of reef coral resistance to future climate change. Science 344, 895–898.

Chiu, W.-D., Cheng, L.-Z. & Ou, H.-C. 1993 Relationship Between the Dispersion of Thermal Effluent and the Tidal Current in the Waters Near the Outlet of the Third Nuclear Power Plant in Southern Taiwan. Journal of Fisheries Society of Taiwan 20, 207–220.

Chou, Y., Lin, T. Y., Chen, C. T. A. & Liu, L. L. 2004 Effects of nuclear power plant thermal effluent on marine sessile invertebrate communities in Southern Taiwan. Journal of Marine Science and Technology 12, 448–452.

Fan, K. L. 1991 The Thermal Effluent Problems of Three Nuclear Power Plants in Taiwan. In Oceanography of Asian Marginal Seas, pp. 393–403.

Hung, T. C. & Huang, C. C. 1998 Ecological survey of coastal water adjacent to nuclear power plants in Taiwan. Chemistry and Ecology

Keshavmurthy, S., Hsu, C.-M., Kuo, C.-Y., Meng, P.-J., Wang, J.-T. & Chen, C. A. C. 2012 Symbiont communities and host genetic structure of the brain coral Platygyra verweyi, at the outlet of a nuclear power plant and adjacent areas. Molecular Ecology 21, 4393–4407.

Keshavmurthy, S., Meng, P.-J., Wang, J.-T., Kuo, C.-Y., Yang, S.-Y., Hsu, C.-M., Gan, C.-H., Dai, C.-F. & Chen, C. A. C. 2014 Can resistant coral-Symbiodinium associations enable coral communities to survive climate change? A study of a site exposed to long-term hot water input. PeerJ 2, e327. (doi:10.7717/peerj.327)

Keshavmurthy, S., Kuo, C.-Y., Huang, Y.-Y., Carballo-Bolanos, R., Meng, P.-J., Wang, J.-T., Chen, C. A. 2019 Coral reef resilience in Taiwan: Lessons from long-term ecological research on the coral reefs of Kenting National Park (Taiwan). Journal of Marine Science and Engineering 388, doi.org/10.3390/jmse7110388.

Lee, H. J., Chao, S. Y., Fan, K. L. & Wang, Y. H. 1997 Tidally induced upwelling in a semi-enclosed basin: Nan Wan Bay. Journal of Oceanography 53, 467-480.

Lee, H.-J., Chao, S.-Y. & Fan, K.-L. 1999 Flood–ebb disparity of tidally induced recirculation Eddies in a semi-enclosed basin: Nan Wan Bay. Continental Shelf Research 19, 871–890.

Kao, K. W., Keshavmurthy, S., Tsao, C. H., Wang, J. T., Chen, C. A .2018 Repeated and Prolonged Temperature Anomalies Negate Symbiodiniaceae Genera Shuffling in the Coral Platygyra verweyi (Scleractinia; Merulinidae). Zoological Studies 57:55. doi:10.6620/ZS.2018.57- 55.

Wellington, G. M., Glynn, P. W., Strong, A. E., Navarrete, S. A., Wieters, E. & Hubbard, D. 2006 Crisis on coral reefs linked to climate change. Eos Trans. AGU 82, 1–5.

Liu, G., Strong, A. E. & Skirving, W. 2003 Remote sensing of sea surface temperatures during
2002 Barrier Reef coral bleaching. *Eos Trans. AGU* **84**, 137–141.

46. Schoepf, V., Stat, M., Falter, J. L. & McCulloch, M. T. 2015 Limits to the thermal tolerance of corals adapted to a highly fluctuating, naturally extreme temperature environment. *Scientific Reports* **5**, 17639.

47. Ferrara, G. B., Murgia, B., Parodi, A. M., Valisano, L., Cerrano, C., Palmisano, G., Bavestrello, G. & Sara, M. 2006 The assessment of DNA from marine organisms via a modified salting-out protocol. *Cellular & molecular biology letters* **11**, 155–160.

48. Mieog, J., van Oppen, M., Cantin, N. & Stam, W. 2007 Real-time PCR reveals a high incidence of *Symbiodinium* clade D at low levels in four scleractinian corals across the Great Barrier Reef; implications for symbiont shuffling. *Coral Reefs* **26**, 449-457.

49. Rasmussen, R. 2001 Quantification on the LightCycler. In *Rapid Cycle Real-Time PCR*, pp. 21–34. Berlin, Heidelberg: Springer, Berlin, Heidelberg.

50. Tay, Y. C., Noreen, A. M. E., Suharsono, Chou, L. M. & Todd, P. A. 2014 Genetic connectivity of the broadcast spawning reef coral *Platygyra sinensis* on impacted reefs, and the description of new microsatellite markers. *Coral Reefs* **34**, 301–311.

51. Miller, K. J. & Howard, C. G. 2004 Isolation of microsatellites from two species of scleractinian coral. *Molecular Ecology Notes* **4**, 11–13.

52. Yang, S. Y., Fong, W. L., Chow, W. S., Zoological, C. H. 2018 (In press) Development of Novel Polymorphic Microsatellite Markers in Catch Bowl Coral, *Isopora palifera* (Scleractinia; Acroporidae) Using Next-generation Sequencing.

53. Schuelke, M. 2000 An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* **18**, 233–234.

54. Peakall, R. & Smouse, P. E. 2012 GenAlEx 6.5; genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics* **28**, 2537–2539.

55. Rice, W. R. 1989 Analyzing Tables of Statistical Tests. *Evolution* **43**, 223–225.

56. Excoffier, L., resources, H. L. M. E. 2010 In press. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564-567.

57. Pritchard, J. K., Stephens, M., Donnelly, P. 2000 Inference of population structure using multilocus genotype data. *Genetics* **155**, 945-959.

58. R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

59. Bay, L. K., Doyle, J., Logan, M. & Berkelmans, R. 2016 Recovery from bleaching is mediated by threshold densities of background thermo-tolerant symbiont types in a reef-building coral. *Royal Society Open Science* **3**, 160322.

60. Lee, J.L., Jeong, H. J., Jang, S. H., Lee, S. Y., Kang, N. S., Lee, K. H., Kim, S. K., Wham, D. C., LaJeunesse, T. C. 2016 Most low-abundance "Background" *Symbiodinium* spp. are transitory and have minimal functional significance for symbiotic corals. *Microb Ecol* **71**, 771-783.

61. Howells, E. J., Abrego, D., Meyer, E., Kirk, N. L. & Burt, J. A. 2016 Host adaptation and unexpected symbiont partners enable reef-building corals to tolerate extreme temperatures. *Global Change Biology* **22**, 2702–2714.

62. Obura, D. O. 2005 Resilience and climate change: Lessons from coral reefs and bleaching in the Western Indian Ocean. *Estuarine Coastal And Shelf Science* **63**, 353–372.
Building Corals. *PLoS ONE* 5, e13258.
Figures

Figure 1. Map showing the reciprocal transplant experiment locations in the Kenting National Park, Taiwan. NPP-OL – Nuclear Power Plant Outlet, NPP-IL – Nuclear Power Plant Inlet, and WLT – Wanlitung.

Figure 2. Seawater temperature (weekly average) trend (A) and DHW (B) at three locations: Red line – NPP-OL, Black line – WLT, and Blue line – NPP-IL, in 2014 and 2015. Typhoons in 2015 are represented as arrows.

Figure 3. Symbiodiniaceae genera trends (A) in five native cores of P. verweyi during the 2014 transplantation experiment and (B) in 30 native cores of P. verweyi during the 2015 transplantation experiment. Each colour block represents one core sampled from one individual colony. In the case of NPP-IL, no samples were collected after July 2015. X = the cores were dead.

Figure 4. Symbiodiniaceae genera trends in bi-monthly sampling of tagged mother colonies at each location in 2014-2016. Each colour block represents one core sampled from one individual colony. In the case of NPP-IL, there were no samples after November 2015.

Figure 5. Bar plot of STRUCTURE. Bayesian clustering analysis for eight loci genotypes among the NPP-OL and WLT P. verweyi populations. This bar plot assumed the number of population K=2. 1,000,000 MCMC runs.
### Table 1 (on next page)

Characteristics of microsatellite loci

Characteristics of eight microsatellite loci for 60 colonies of *P. verweyi* collected at NPP-OL and Wanlitung.
Table 1. Characteristics of eight microsatellite loci for 60 colonies of *P. verweyi* collected at NPP-OL and Wanlitung.

| locus | Primers sequences | repeats | Size of alleles | Tm (℃) | No. alleles | Hₑ  |
|-------|-------------------|---------|----------------|--------|------------|-----|
| PV9   | F:†CAACTTAAATGGTATCATCGTG  
|       | R: GTGCCCTATTTTATGTGACAA   | (AG)₂₆ | 140-168        | 50     | 9          | 0.85|
| PV19  | F:†TAGTCAGTGCGATCGAGATG  
|       | R: CTCATTTCCTCCTAAGCTTTC | (TG)₃₀ | 161-197        | 53     | 16         | 0.83|
| PV22  | F:†TCACCTGCTATAACCTTCCTCTC  
|       | R: TCCACCTCTCCTCCACTAGTTATC | (TG)₁₂ | 140-164        | 50     | 10         | 0.86|
| PV56  | F:†TGGACTCGTCAATCCACTATC  
|       | R: GCTAGCAGTGATCAACAGAT   | (TC)₁₄ | 144-166        | 50     | 6          | 0.75|
| PV57  | F:†ACAGACACAGACAGACACAGAA  
|       | R: CAGTTCACCTGTCCATTGA    | (TC)₁₄ | 103-119        | 50     | 6          | 0.60|
| Plsi4.0 2  | F:†ACAATTCGGATATGTAGC  
|       | R: GTTTCTTTGGTTGTTGTCTTC | (AAAC)₁₁ | 136-170    | 50     | 14         | 0.85|
| Plsi4.2 4  | F:†TTATCTTGGTCGAGACACAGA  
|       | R: GTTTGACAACCTCTAATGAGGTACAG | (ACAG)₁₀ | 126-158     | 59     | 9          | 0.77|
| PD31  | F:†GACAATGTAATGTAATCGTG  
|       | R: †CTGTTAGAGTATCTAGTGCTTGAC | (CCAT)₇ | 156         | 56     | 7          | 0.54|

† primer attached M13 (-21) tail (5’- TGT AAA ACG ACG GCC AGT -3’)(18 bp)
Figure 1

Map showing the reciprocal transplant experiment locations in the Kenting National Park, Taiwan.

Map showing the reciprocal transplant experiment locations in the Kenting National Park, Taiwan. NPP-OL – Nuclear Power Plant Outlet, NPP-IL – Nuclear Power Plant Inlet and WLT-Wanlitung.
Figure 2

Seawater temperature (weekly average) trend

Seawater temperature (weekly average) trend (A) and DHW (B) at three locations: Red line - NPP-OL, Black line - WLT and Blue line - NPP-IL, in 2014 and 2015. Typhoons in 2015 are represented as arrows.
Figure 3

Symbiodiniaceae genera trends

Symbiodiniaceae genera trends (A) in five native cores of *P. verweyi* during the 2014 transplantation experiment and (B) in 30 native cores of *P. verweyi* during the 2015 transplantation experiment. Each colour block represents one core sampled from one individual colony. In the case of NPP-IL, no samples were collected after July 2015. X = the cores were dead.
Figure 4

Symbiodiniaceae genera trends

Symbiodiniaceae genera trends in bi-monthly sampling of tagged mother colonies at each location between 2014-2016. Each colour block represents one core sampled from one individual colony. In the case of NPP-IL, there were no samples after November 2015.
Manuscript to be reviewed

Presence of Symbiodiniaceae genera (%)

- Cladocopium sp.
- Durusdinium sp.

Mother colonies:
- NPP IL
- WLT
- NPP OL

- Nov 2014
- Jan 2015
- Mar 2015
- May 2015
- Jul 2015
- Oct 2015
- Nov 2015
- Jan 2016
- Apr 2016
Figure 5

Bar plot of STRUCTURE

Bar plot of STRUCTURE. Bayesian clustering analysis for eight loci genotypes among the NPP-OL and WLT P. verweyi populations. This bar plot assumed the number of population K=2. 1,000,000 times MCMC runs.