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

Shashank Keshavmurthy [1] , **Hwee-Sze Tee** [1,2] , **Kuo-Wei Kao** [1,3] , **Jih-Terng Wang** [Corresp., 4] , **Chaolun Allen Chen** [Corresp. 1,3,5]

[1] Biodiversity Research Center, Academia Sinica, Taipei, Taiwan

[2] Taiwan International Graduate Program, International Internship Program, Academia Sinica, Taipei, Taiwan

[3] Institute of Oceanography, National Taiwan University, Taipei, Taiwan

[4] Department of Oceanography, National Sun Yat-Sen University, Kaoshuing, Taiwan

[5] Department of Life Science, Tunghai University, Taichung, Taiwan

Corresponding Authors: Jih-Terng Wang, Chaolun Allen Chen
Email address: jtw@tajen.edu.tw, cac@gate.sinica.edu.tw

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 6ºC-weeks in 2014 and 4ºC-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 conditionsby 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.

1   **Specificity trumps flexibility—location-based stable associations between Symbiodiniaceae**

2   **genera and *Platygyra verweyi* (Scleractinia; Merulinidae)**

3   Shashank Keshavmurthy[1#], Hwee-Sze Tee[1,2#], Kuo-Wei Kao[1,3], Jih-Tern Wang[4✉], Chaolun Allen

4   Chen[1,3,5✉]

6   [1]Biodiversity Research Center, Academia Sinica, Nangang, Taipei, Taiwan

7   [2]Taiwan International Graduate Program, International Internship Program, Academia Sinica,
8   Nangang, Taipei, Taiwan

9   [3]Institute of Oceanography, National Taiwan University, Taipei, Taiwan

10   [4]Department of Oceanography, National Sun Yat-Sen University, Taiwan

11   [5]Department of Life Science, Tunghai University, Taichung, Taiwan

13   #: equal contribution

14   Corresponding authors: Chaolun Allen Chen, E-mail: cac@gate.sinica.edu.tw;

15   Jih-Tern Wang, E-mail: jtwtaiwan@gmail.com

17 **Abstract**

18 This study monitored symbiont communities bi-monthly in native coral cores used in a reciprocal

19 transplantation of the coral *Platygyra verweyi* over two years (2014-2016) and samples of mother

20 colonies from three locations with variable thermal regimes; our results show that associating with

21 multiple Symbiodiniaceae genera (*Cladocopium* spp. and *Durusdinium* spp.) is not a prerequisite

22 for symbiont shuffling. *Platygyra verweyi* associates with certain Symbiodiniaceae genera based

23 on location. Results of quantitative real-time PCR indicated small-scale temporal changes in

24 Symbiodiniaceae genera compositions from 2014-2016; however, these changes were not enough

25 to invoke shuffling or switching, despite degree heating weeks exceeding 6ºC-weeks in 2014 and

26 4ºC-weeks in 2015, which usually resulted in substantial coral bleaching. Microsatellite analysis

27 of the *P. verweyi* host showed no genetic differences among the study locations. Our results

28 suggest that *P. verweyi* undergoes long-term acclimatization and/or adaptation based on

29 microgeographic and local environmental conditions by altering its combinations of associated

30 Symbiodiniaceae. Results also suggest that shuffling might not be as common a phenomenon as it

31 has been given credit for; corals thrive through specific associations, and many corals could still

32 be vulnerable to climate change-induced stress, despite being promiscuous or able to associate

33 with rare and background Symbiodiniaceae genera.

35 **Key Words:** Reciprocal transplantation, Thermal histories, Degree heating weeks,
36 Symbiodiniaceae, Nuclear power plant
37

38 **Background**

39 Corals in reefs around the world have been facing rapid declines in health over the past several

40 decades due to increased and prolonged occurrences of climate change-induced seawater

41 temperature anomalies, which often pass their threshold limits [1-3]. A common manifestation of

42 this stress is coral bleaching due to the breakdown of coral-Symbiodiniaceae associations [4].

43 Moreover, corals have the potential to acclimate to climate change-induced stressors—over a short

44 period of time (single generation)—through phenotypic plasticity or associating with specific

45 combinations of stress resistant Symbiodiniaceae genera through natural selection; this may be the

46 overriding determinant of their survival [5]. However, a beneficial association between a coral

47 host and Symbiodiniaceae is a complex and holistic process that depends on whether the

48 relationship that the coral host has with the Symbiodiniaceae genera is specific or flexible [6-8].

49 Corals are known to associate with a wide range of Symbiodiniaceae genera. There are nine genera

50 of Symbiodiniaceae, and each has its own characteristic traits that help its coral host survive in a

51 wide range of environmental niches [9]. Studies have shown that symbiosis between coral hosts

52 and different Symbiodiniaceae genera contributes to the divergence in coral thermal tolerance

53 under different environmental conditions [7,10]. For instance, species of *Durusdinium* are

54 considered to be heat tolerant [6,11-13],; most species *Cladocopium* are stress sensitive but several

55 are relatively stress tolerant (*e.g. in- hospite Cladocopium* C15, *Cladocopium thermophilum*)

56 [14,15]. *Durusdinium*-associated corals are also known to inhabit reef environments that

57 experience large fluctuations in surface seawater temperature [7,16,17] and be more resilient to

58 heat-treatment experiments [18].

59 One widely-known mechanism that helps some corals acclimate to stressful environments is

60 shuffling and/or switching their associated Symbiodiniaceae genera [19-25]. It has been proposed

61 that coral hosts adjust to increasing seawater temperatures using switching—which involves

62 existing *Symbiodinium* being expelled and replaced by novel *Symbiodinium* from the

63 environment—and shuffling between stress-sensitive (generally *Cladocopium* sp.) and stress-

64 resistant types (generally *Durusdinium* sp.) in the existing symbiont communities [19, 20, 26, 27].

65 Shuffling between Symbiodiniaceae genera has been found to benefit some coral species [11, 12]

66 because increasing the abundance of stress-tolerant Symbiodiniaceae genera in a multi-symbiont

67 association helps corals withstand above-threshold seawater temperature anomalies [16-18]. A

68 transition from thermally sensitive to tolerant dominant symbionts can increase the likelihood that

69 corals survive thermally-induced bleaching [28]. However, a later study [29] argued that not all
70 corals can change their symbionts, because the mechanism of shuffling requires that a coral species
71 hosts multiple Symbiodiniaceae genera (at least one stress tolerant and one stress resistant). Some
72 coral species have the ability to fluctuate between Symbiodiniaceae genera on a temporal scale
73 (e.g. [30]). The occurrence of multiple Symbiodiniaceae genera at low densities might lead to
74 either shuffling or switching to beneficial Symbiodiniaceae genera over time [30]. In some cases,
75 the coral host may revert back to its original composition of either a single dominant
76 Symbiodiniaceae species or multiple species/genera (see [31]). However, there are also many cases
77 in which the host maintains stable symbiosis with a particular Symbiodiniaceae genus, irrespective
78 of environmental perturbations (see [31,32]). This acclimatization mechanism, although limited,
79 may help corals survive the effects of ocean warming in the near future [20, 33].

80 In order to assess whether associating with multiple Symbiodiniaceae is in itself enough for
81 shuffling to happen, we analysed coral samples collected over time from locations with different
82 thermal regimes to determine how location influences corals' abilities to shuffle and subsequently
83 survive. Nanwan, Kenting National Park, Taiwan is a reef site located southwest of the third
84 Nuclear Power Plant Outlet (NPP-OL) that has been affected by the continuous discharge of
85 thermal effluent flowing directly into the existing coral community as a result of the near-shore
86 current [34] since the power plant opened in 1984. NPP-OL has seawater temperatures similar to
87 those predicted for oceans around the world by 2050 [1], making it an ideal location to conduct
88 studies related to the effects of climate change. Nuclear power plant -OL has significantly different
89 community compositions and settlement patterns compared to other sites such as the Nuclear
90 Power Plant Inlet (NPP-IL) [35], suggesting that the thermal effluent has had a great impact on its
91 benthic invertebrate and fish communities. Corals present at NPP-OL have also experienced
92 several bleaching events over time (see [36,37]). The coral communities in the shallow water (3
93 m) are dominated by thermally tolerant symbiont types [30,38,39]. The increasing prevalence of
94 stress-tolerant *Durusdinium* sp. at reef sites closer to NPP-OL reflects the consequences of long-
95 term thermal effects; this prevalence also makes NPP-OL an ideal site to study holobiont dynamics
96 under thermal stress, although other physical differences (temperature fluctuations, upwelling, and
97 internal waves; see [40]) between NPP-OL and other sites could also be involved [30,38,39,41,42].

98   In this study, we collected data from *in situ* reciprocal transplant experiments (RTE) on nubbins

99   of *P. verweyi* collected from NPP-OL, NPP-IL, and Wanlitung (WLT) in Kenting National Park

100   (KNP) between 2014-2016 (see [43]). Using the samples from each location of origin (tagged

101   mother colonies and native cores used in the transplant experiment), we tested whether corals in

102   their native environments will undergo shuffling over time and associate with favourable

103   Symbiodiniaceae genera.

104

105   **Methods**

106   Samples used in the experiment were collected with permission from the Kenting National Park

107   headquarters (permit numbers 1040008112 and 1040002080).

108

109   *Study area and coral specie*s

110   All experiments and sampling were carried out at three locations: Nuclear Power Plant Outlet

111   (NPP-OL), Nuclear Power Plant Inlet (NPP-IL), and Wanlitung (WLT) in Nanwan, southern

112   Taiwan (Fig. 1). NPP-OL (21°55'54.4"N, 120°44'42.7"E) and NPP-IL (21°57'20.3"N,

113   120°45'14.2"E) are located within Nanwan in the Kenting National Park (KNP), Taiwan, and WLT

114   (21°59'41.0"N 120°42'19.6"E) is located on the west coast of KNP, approximately 12 km from the

115   nuclear power plant area. Due to the tidally-induced upwelling in Nanwan [41], the maximum

116   daily seawater temperature fluctuation at NPP-OL and NPP-IL can exceed 8ºC in the summer

117   season. In this study, NPP-IL and WLT were both included to assess the potential role of thermal

118   variability on *P. verweyi* facing prolonged thermal stress. The massive coral species *P. verweyi*

119   generally occurs in shallow water (2-4 m) and was found to associate with the Symbiodiniaceae

120   genera *Cladocopium* sp. and/or *Durusdinium* sp. in KNP [38].

121

122   *Coral samples*

123   Two types of samples were used in the experiment: the "mother colonies" were the original

124   colonies (from the 2014-2016 transplantation experiment), and the "native cores" were samples

125   cut from a mother colony and kept at the same location.

126   Tagged mother colonies at each location (five from NPP-IL in 2014, eight each from NPP-OL and

127   WLT from 2014 to 2016) were sampled approximately every two months throughout the

128   experimental period. During each sampling, a piece (2 cm diameter core) of coral was cut and

129   fixed in 95% Ethanol for DNA extraction and qPCR analysis.

130   Samples were taken from the coral cores in the racks used in the transplantation experiment (see

131   [43] for detailed explanation of the transplantation experiment). All samples were taken from the

132   set of control racks installed at each location from the reciprocal transplant experiment, and hence

133   referred to as "native cores". Five native cores (one from each colony) from each rack in 2014 (at

134   all three sites) and one piece (2 cm in diameter) from each of the 30 cores in 2015 (from the racks

135   at NPP-OL and WLT) were sampled at each location. Sampling for the 2014 set was carried out

136   in April and September of 2014 and January, March, May, July, September, and November of

137   2015. Sampling for the 2015 set was carried out in April, September, and November of 2015 and

138   January and April of 2016. All samples were fixed in 95% Ethanol for DNA extraction and qPCR

139   analysis.

140   *Seawater temperature*

141   The seawater temperature was recorded *in situ* at 30-minute intervals using data loggers (HOBO;

142   Pendant™, USA) deployed underwater near the transplant racks (1-2 m) at each study site. The

143   raw temperature data were transformed into Degree Heating Weeks (DHW) [44,45] to assess both

144   the intensity and duration of the thermal stress for each experiment group. Although this indicator

145   is typically used to monitor large-scale bleaching, it was also used in another study to assess the

146   cumulative thermal stress on heat-treated corals, but daily [46]. DHW was calculated as follows.

147   First, the weekly mean temperature for each study site was calculated from raw temperature data.

148   Second, the maximum of the monthly mean temperatures (MMM; NPP-OL = 29.63ºC; WLT =

149   29.28ºC; NPP-IL = 28.37ºC) was obtained from data loggers (HOBO; Pendant™, USA) deployed

150   at each study location. Finally, the weekly mean temperature was subtracted from MMM to

151   determine temperature anomalies; only temperatures at least 1.0ºC above the MMM within the

152   previous 12 weeks were considered anomalies and summed to obtain the DHW. The conceptual

153   calculation equation is listed below

154    $$DHW_{TWO} = \sum[(T_{NPP-OL} - MMM_{WLT}) \geq 1°C]$$

155   where T_{NPP-OL} is the weekly mean temperature at NPP-OL, MMM_{WLT} is the MMM of WLT, etc.

156   The projections of DHW in Nanwan were obtained from [33] using a 1º × 1º resolution grid of reef

157   cells located in southern Taiwan.

158

159   *DNA extraction*

160   DNA extraction was carried out using a salting-out method modified from [47]. Coral tissue was

161   lysed overnight in a 2-mL Eppendorf tube with 200 µL of lysis buffer [0.25 M Tris, 0.05 M EDTA

162   at pH 8.0, 2% sodium dodecylsulfate (SDS) and 0.1 M NaCl] and 10 µL of 10 mg/mL proteinase

163   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

164   sample was mixed by carefully inverting the tube. The solution was then transferred to a 2-mL

165   collection tube containing a DNA spin column (Viogene, USA) and centrifuged at 8000 rpm for 1

166   min. The lysate was washed twice with 500 μL of ethanol (70%) by centrifuging at 8000 rpm for

167   1 min at each step, with an additional centrifugation step at 8000 rpm for 3 min to dry the spin

168   column. The column was dried further at 37°C for 15 min, then the DNA was eluted with 50 μL

169   of preheated (65ºC) 1X TE buffer, with a final centrifugation at 15000 g for 3 min. The quality of

170   genomic DNA was checked using a 1% agarose gel. The concentrations of genomic DNA were

171   determined using NanoDrop 2000 (Thermal Scientific, USA).

172   *Real-time quantitative PCR*

173   The copy numbers of *Cladocopium* sp. and *Durusdinium* sp. in *P. verweyi* samples were

174   determined under a LightCycler® 480 Instrument II (Roche, Switzerland) using a protocol

175   modified from [48]. Each 10 μL qPCR reaction consisted of 5 μL 1x SYBR Fast Master Mix, 0.5

176   μL UF primer (2 nM/μL), 0.5μL CR or DR primer (2 nM/μL), 7.5 μL ddH$_2$O, and 2.5 μL DNA

177   templates (equal to 1 ng of genomic DNA). The following primer sets were used: ITS1 clade C-

178   specific reverse primer (CR) 5-AAGCATCCCTCACAGCCAAA-3, clade D-specific reverse

179   primer (DR) 5-CACCGTAGTGGTTCACGTGTAATAG-3, and universal forward primer (UF) 5-

180   AAGGAGAAGTCGTAACAAGGTTTCC-3 [12]. Each sample was run in triplicate (technical

181   replicates), as was a no-template control (NTC) with ddH$_2$O. Plasmid standard curves were run in

182   duplicate with *P. verweyi* samples to quantify the copy numbers of each symbiont. Plasmid

183   standard curves were generated using PCR products from *Cladocopium* sp. and *Durusdinium* sp.,

184   which were ligated into pGem®-T Easy vectors (Promega, USA), transformed, and amplified using

185   *E. coli*. Copy numbers of final products were calculated by first quantifying the concentration of

186   plasmid DNA through NanoDrop 2000 (Thermal Scientific, USA), then dividing by the mass of

187   the plasmid [mass of each plasmid copy = 3015 bp (vector) + 100 bp (inserted PCR product) x

188    $1.096e^{-21}$ g/bp = 3.4×10^{-18} g], and finally multiplying by the plasmid DNA template volume (2.5

189   μL) in each reaction. Serial dilutions of 1:10 from 3×10^6 and 30 copies of the plasmid standard

190   containing *Cladocopium* sp. and *Durusdinium* sp. sequences, respectively, were generated in the

191   end. The qPCR cycling settings were: 40 two-step cycles of 15 s at 95ºC and 1 min at 60ºC. Melting

192   curves were generated by starting at 60ºC and increasing the temperature with a ramp speed of

193   0.11ºC/s until it reached 95ºC. Fluorescence data were collected after each annealing step, and five

194 readings were collected every second during the melting curve analysis. Crossing points (Cp) were

195 determined by Light Cycler 480 software version 1.5 (Roche, Switzerland) using the second

196 derivative method, which represents the cycles with the maximum number of fluorescence signals

197 in each sample [49]. Samples with Cp values that varied from the other two technical replicates by

198 1 were excluded from analysis. Samples were re-run if all Cp values of technical replicates varied

199 from one another by 1. Since high variation occurred in Cp values (varied more than 1) within

200 technical replicates of each sample when Cp>34, the cut-off cycle was set to 34 to avoid false

201 positives caused by the formation of non-specific fluorescence. Average copy numbers of

202 symbiont clades C and D were obtained individually, and the formula for determining relative

203 symbiont abundance in this study is listed below, following a correction suggested by [48]:

204 (Clade D copy numbers / 3) / [(Clade D copy numbers / 3) + Clade C copy numbers]

205

206 *Population genetics analysis of* P. verweyi

207   To demonstrate the presence of a genetic structure in *P. verweyi* at NPP-OL and WLT, eight

208 polymorphic microsatellite loci were used to examine 30 *P. verweyi* colonies (including

209 transplanted colonies) found within two transplanted sites. Three published microsatellite tetramer

210 markers developed from *P. sinensis* [50] and *P. daedalea* [51] were used. Another five

211 microsatellite dimer markers specific to *P. verweyi* were developed by Next Generation

212 Sequencing approaches [52]. Eight microsatellite markers were amplified following the effective

213 universal fluorescent labeling method [53]. Amplifications performed using 25 μL reactions

214 contained 10 ng of DNA template, 1X of VeraSeq Buffer II (Qiagen Beverly, USA), 0.5U of

215 VeraSeq 2.0 high-fidelity DNA polymerase (Qiagen Beverly, USA), 0.2 mM of dNTP mix, 0.08

216 μM of specific forward primer-attached M13 (-21) tail (5'- TGT AAA ACG ACG GCC AGT -3')

217 (18 bp), 0.2 μM of specific reverse primer, and 0.2 μM TAMRA-labelled universal M13 (-21)

218 primer (5'- TGT AAA ACG ACG GCC AGT -3') [53]. The PCR conditions were: 1 cycle at 98ºC

219 for 30 s; 25 cycles of 98ºC for 10 s, specific primer annealing temperature (Table 1) for 30 s, and

220 72ºC for 30 s; followed by 10 cycles of fluorescent-labelled M13 amplification: 98ºC for 10 s,

221 53ºC for 30 s, 72ºC for 30 s; and a final elongation of 10 min at 72ºC. For microsatellite

222 genotyping, samples were electrophoresed on 5% urea denaturing polyacrylamide gels using the

223 Gel-Scan 3000™ real-time DNA fragment analysis gel Electrophoresis System (Corbett Robotics,

224 Australia). Allele size was detected by the software Gene Profiler 4.05 (Scanalytics) with the

225 internal lane size standard (GeneScan™-350 TAMRA™, Applied Biosystems). Characteristics of

226 microsatellite loci—such as number of alleles and mean observed and expected heterozygosities—

227 were calculated using GenAlEx v.6.502 [54]. Genepop was used on the web to test for linkage

228 disequilibrium and significant departure from Hardy-Weinberg equilibrium (HWE). None of the

229 loci showed HWE deviation or linkage disequilibrium after Bonferroni correction [55]. Population

230 differentiation was inferred using ARLEQUIN v3.5 [56]. Inference population genetic structure

231 was estimated using a Bayesian clustering approach implemented in STRUCTURE v.2.3.4 [57].

232 The admixture model and allele frequency correlation were used. Values of number of genetic

233 clusters (K) from 1 to 2 were tested by running three replicate simulations per K with 1,000,000

234 Markov chain Monte Carlo repetitions and 100,000 burn-in iterations.

235 *Statistical analysis*

236 All statistical analyses in this study were performed in R version 3.1.1. [58]. Differences in daily

237 mean seawater temperatures and daily seawater temperature fluctuations between sites were tested

238 using Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni adjusted p-values.

239

240   **Results**

241   *Seawater temperature*

242   Weekly average seawater temperatures were plotted from the data collected by data loggers from
243   2014 and 2106. Both monthly and daily average seawater temperatures at NPP-OL were
244   significantly higher (2.0–3.0ºC) than at adjacent locations (Fig. 2A). In 2014, the average summer
245   (June to August) daily seawater temperature at NPP-OL (30.11 ± 1.07 SDºC) was different from
246   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
247   °C; p<0.001). In 2015, the average summer daily seawater temperature at NPP-OL (29.77 ± 1.12
248   SD °C) was different from that at WLT (29.52 ± 0.52 SD °C; Wilcoxon rank sum test, W=5097,
249   p<0.05). The daily seawater temperature fluctuation at NPP-OL (2.38 ± 1.04 SD °C) was different
250   from that at WLT (1.57 ± 0.70 SD°C) (Wilcoxon rank sum test, W=655557, p<0.001). The heating
251   event (≥ 30°C) at NPP-OL occurred for a longer time each day than at WLT.

252   The daily seawater temperature fluctuation at NPP-OL (2.23 ± 1.00 SD °C) was different from
253   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
254   fluctuations did not vary between WLT and NPP-IL (p=1.000). During the summer, however, the
255   daily seawater temperature fluctuation at both NPP-OL and NPP-IL was more than 7°C (maximum
256   9.12°C at NPP-OL and 7.19°C at NPP-IL). The daily heating event (≥30°C) occurred for the
257   longest time at NPP-OL.

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

265

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

267   The results of the real-time qPCR analysis of the samples from the 2014 experiment (Fig. 3A)

268   indicated that, until January 2015, the symbiont communities in the native cores of NPP-OL were

269   dominated by *Durusdinium* spp. In March 2015, the relative proportions of symbionts changed:

270   *Cladocopium* spp. were present at various percentages (range: 1-21%; mean: 6%; Fig. 3A), and

271   *Durusdinium* spp. accounted for the rest. Symbiont dynamics in the core with 21% *Cladocopium*

272   spp. did continue to fluctuate (13% in May; 18% in July and September; Fig. 4). However, this

273   core was dead by November 2015. All remaining cores survived, fluctuating between 1-5% (mean:

274   2%) *Cladocopium* spp. for the entire experiment.

275   In the 2015 experiment (Fig. 3B), the symbiont community in the native core samples from NPP-

276   OL were dominated by *Durusdinium* spp. However, qPCR suggested that the cores in the samples

277   from April 2016 were associated with *Cladocopium spp.* in addition to the already present

278   *Durusdinium* spp. Previously undetected levels of *Cladocopium* spp. increased enough to be

279   detected by qPCR. Cores were found to contain 3-61% (mean: 22.6%) *Cladocopium* spp. in April

280   2016.

281   Bimonthly sampling of the mother colonies revealed that *P. verweyi* was specific with respect to

282   which Symbiodiniaceae genera it associated with (Fig. 4). Colonies sampled from NPP-OL

283   showed changes in associated Symbiodiniaceae, meaning that none of the 30 colonies analysed

284   were associated with 100% *Durusdinium* spp. throughout the entire study period. However, the

285   fluctuation between *Durusdinium* spp. and *Cladocopium* spp. was not large. Colonies were found

286   to host 91-100% *Durusdinium* spp. (seven of the nine colonies)—one colony hosted 50 and 80%

287   at two sampling times—with the exception of one colony, which associated with 72 and 99%

288   *Durusdinium* spp. in November 2014 and January 2015, respectively. However, this colony

289   changed its relative proportion of symbionts to 100% *Cladocopium* spp. in March 2015, and then

290   reverted to 97% *Durusdinium* spp. in May 2015. This was the only colony that had a large

291   fluctuation in associated Symbiodiniaceae between the two Symbiodiniaceae genera.

292

293   *Temporal variation in Symbiodiniaceae genera associated with* P. verweyi *at WLT*

294   In the 2014 experiment samples from WLT, a predominance of *Cladocopium* spp. was observed

295   in the majority of the cores (Fig. 3A), with the exceptions of two cores found dead from March

296   2015 onwards and one colony that reverted to *Durusdinium* spp. (83%).

297   In 2015, cores from WLT were mainly associated with *Cladocopium* spp., ranging from 5-99%

298   (mean: 31%) (Fig. 3B). Five of the 30 cores were associated with *Durusdinium* spp. throughout

299   the experimental period. However, in November 2015, 12 of the 30 cores were associated with

300   *Durusdinium* spp. (range: 1-87%; mean: 32%). Three cores at WLT that were mainly associated

301   with *Cladocopium* spp. were dead by the end of the sampling period in April 2016 (Fig. 3B).

302   In the case of the WLT bimonthly analysis of the samples from the mother colonies, seven of the

303   eight colonies analysed were associated only with *Cladocopium* spp., except for one colony, which

304   also associated with *Durusdinium* spp. (2% in November, 2014; 1% in July, 2015; and 2% in April,

305   2016) in addition to *Cladocopium* spp. (Fig. 4).

306

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

308   In 2014, all the cores at NPP-IL were found to be predominantly associated with *Cladocopium*

309   spp. throughout the experimental period. None of the cores were associated with *Durusdinium* spp.

310   (Fig. 3A).

311   With respect to the bimonthly analysis of the samples collected from the mother colonies, again

312   all the samples were predominantly associated with *Cladocopium* spp. throughout the sampling

313   period. However, some colonies did show the presence of *Durusdinium* spp. (1% in March, May,

314   and November; 29% in October, 2015; Fig. 4).

315

316   *Microsatellite analysis of host samples from NPP-OL and WLT*

317   Microsatellite analysis revealed that a total 60 *P. verweyi* colonies (30 each from NPP-OL and

318   WLT) exhibited six to 16 alleles per locus for all eight microsatellite loci, with a mean expected

319   heterozygosity of 0.756 ± 0.122 (Table 1). Average gene diversity of *P. verweyi* at NPP-OL across

320   eight loci was 0.608 ± 0.393; the average for populations at WLT was 0.657 ± 0.460. The pairwise

321   genetic differentiation F_{st} value between the two sites was -0.00814, and the p-value showed no

322   significant difference. The genetic structure analysis by Bayesian clustering between NPP-OL and

323   WLT also showed no significant differences and no genetic isolation between the two locations

324   (Fig. 5).

325

326 **Discussion**

327 The present study shows spatial variation but specificity in the dominant Symbiodiniaceae genera

328 in the coral *P. verweyi,* and this may be related to local thermal histories. What was seen in *P.*

329 *verweyi* is an almost stable association with a dominant Symbiodiniaceae genus at each sampling

330 time from 2014 to 2016. A fluctuation was observed between Symbiodiniaceae in native cores

331 and/or mother colonies, but this was not a general phenomenon. There was a certain level of

332 temporal and spatial fluctuation in the native coral cores and tagged mother colonies. For example,

333 in the 2014 experiment, some cores at NPP-OL (nubbins here are associated with *Durusdinium*

334 spp.) did acquire low percentages of *Cladocopium* spp. (in March 2015) relative to the already

335 present *Durusdinium* spp. (Fig. 3A). A similar fluctuation was seen in one core from WLT, which

336 was found to have acquired up to 83% *Durusdinium* spp. However, none of the cores at NPP-IL

337 showed any fluctuation (Fig. 3A). A similar pattern was observed in the tagged mother colonies,

338 with low levels of fluctuation and Symbiodiniaceae genera acquisition (Fig. 4).

339 Considering the differences in seawater temperature regimes among the three locations (Fig. 2A),

340 this study assumed that native cores and mother colonies would show different levels of

341 Symbiodiniaceae genera shuffling through time. However, the results showed that one

342 Symbiodiniaceae genus was always dominant in each sample (Fig. 3, 4). For example, samples at

343 NPP-OL were always dominated by *Durusdinium* spp. Such preference could be because corals at

344 NPP-OL are exposed to long-term seawater temperature stress at shallow depths (1-5 m) and hence

345 are naturally acclimatized to an association with *Durusdinium* spp.

346 Association with a particular dominant symbiont could help *P. verweyi* dominate shallows at

347 various locations in KNP. During the experiment and sampling periods (2014-2016), there was

348 one major bleaching event (2014) and several typhoons (2015). Irrespective of the type of

349 symbiont *P. verweyi* associated with, none of the corals experienced any clear bleaching—

350 although some degree of paling of the tissue was observed. Increased seawater temperatures in

351 2014 resulted in DHWs of 6, 2.5, and 1ºC-weeks at NPP-OL, WLT, and NPP-IL, respectively [43].

352 DHW in 2015, however, was below 4ºC-weeks in both NPP-OL and WLT (Fig. 2B). The lower

353 DHW values in 2015 could be attributed to the typhoons that occurred that year: the southern coast

354 of Taiwan was hit by three typhoons in the summer, July – September 2015, resulting in the

355 seawater temperature cooling. Such intense changes in seawater temperature conditions might

356 have resulted in corresponding fluctuations in associated Symbiodiniaceae genera in some cores

357 or tagged mother colonies (Fig. 3, 4). Temperature anomalies found in 2014, with high DHWs,

358 were not enough to yield any pronounced shuffling or switching in this coral.

359 Our observations beg the following questions: how common is shuffling in corals that can

360 associate with two different symbionts, and does flexibility in symbiont associations via shuffling

361 aid corals under stress? Studies have pointed out that corals often associate with two or more

362 symbionts, with one being dominant and others present at low proportions (<5%, background

363 symbiont) [8]. Therefore, corals have potential to shuffle by regulating their proportions of

364 background symbionts when faced with unfavourable conditions. In this study, we detected

365 symbionts at abundances as low <1%; however, such low concentrations might not be a

366 prerequisite for shuffling or even switching. Also, the recent use of NGS amplicon sequencing has

367 uncovered a rare biosphere with the potential to shuffle and/or switch between different

368 Symbiodiniaceae genera. For example, [31] investigated the Symbiodiniaceae rare biosphere in

369 two Pocilloporid species from Lord Howe Island in the Great Barrier Reef over two years. Their

370 results showed that, following two consecutive bleaching events, the species shuffled and became

371 associated with new Symbiodiniaceae genera (most <1% of the relative abundance, with one

372 resistant type reaching 33% of the relative abundance).

373 On the other hand, [59] suggested that a pre-stress *Symbiodiniaceae* (D:C) ratio of <0.003 limits

374 the ability of corals to survive bleaching after shuffling. And another study [60] showed that

375 variation in the presence and abundances of background or low percentages of symbionts in corals

376 is not necessarily related to shuffling, and may have little or no importance in coral physiology.

377 Future studies need to examine the physiological role of those rare biospheres in terms of

378 supporting corals' responses to stress.

379 It may be argued that specificity to a particular Symbiodiniaceae genus depends on the host. We

380 performed genetic analyses on the host using mitochondrial and nuclear markers in a previous

381 study [38] and microsatellite markers in this study. The results showed no genetic difference in the

382 host between locations. This suggests that other factors influence the type of association we see in

383 this coral, including possible local and microgeographic adaptations to seawater temperature. It

384 may be that using more advanced techniques would help uncover prevalent genetic differences

385 between hosts from two locations in KNP, as was shown in a recent study—[61], which showed a

386 clear genetic difference in *P. daedalea*-associated Symbiodiniaceae between Oman and Abu Dhabi

387 in the Persian Gulf, and hence demonstrated a difference in their eco-physiological behaviour.

388   Both macro- and micro-environmental differences between locations could dictate
389   Symbiodiniaceae genera associations in *P. verweyi*. For example, typhoons and upwelling or
390   fluctuating temperatures in shallow reef areas could raise the thermal tolerance of coral, as could
391   the influence of fluctuating environmental factors such as tidal exposure [62]. Although we
392   demonstrated that Symbiodiniaceae mediate *P. verweyi* acclimatization, we cannot rule out the
393   possibility that mutations to the host itself and natural selection lie behind this species' ability to
394   adapt to a particular condition. The effect of micro-environment might also explain the difference
395   seen in the Symbiodiniaceae genera association between the native cores and mother colonies.
396   Specificity towards a particular Symbiodiniaceae genus was more apparent in the mother colonies
397   (Fig. 4). Native cores, due to their small size and hence propensity toward stress, showed more
398   flexibility in their associated Symbiodiniaceae genera (Fig. 3 A, B) [also see the results in 43].
399   We hypothesize that combinations of *P. verveyi* and Symbiodiniaceae genera tend to be specific
400   due to the differences in thermal histories, temperature variations, and hosts favouring one
401   dominant symbiont rather than shuffling. This could have a negative impact on coral exposed to
402   above-threshold thermal anomalies. For example, [43] observed that, when *P. verveyi* nubbins
403   were reciprocally transplanted between NPP-OL and WLT, those from WLT that were associated
404   with *Cladocopium spp.* did not tolerate long-term changes in temperature levels or daily
405   fluctuations. The transplanted nubbins did not survive, even after shuffling. In contrast, nubbins at
406   NPP-OL survived and actually fared well in the more stable and lower-temperature environment
407   of WLT. In fact, they also showed an increase in growth over time, all the while associating with
408   *Durusdinium* sp*p.,* and did not shuffle to *Cladocopium* spp. even though they could have. Results
409   from this study and symbiont association data and host population genetics at a micro-geographic
410   scale (see [38]) hint towards local adaptation in *P. verweyi*.
411

Conclusions

413   Shuffling is not a simple and straightforward way for corals to cope with the effects of climate
414   change, but is in fact a complex process governed by host-symbiont specificity as well as local
415   macro- and micro-environmental conditions. Being flexible [8] is a good strategy, but specificity
416   is also a norm. In other words, a mere increase in temperature above the threshold limit is not
417   enough to invoke shuffling, even if a coral host has the capacity to associate with multiple
418   symbiont partners (see [43]). While it is popular to be optimistic that shuffling or switching

419 between Symbiodiniaceae genera is a way for corals to survive frequent above-threshold seawater
420 anomalies, we should be cautious, as not all coral species appear to be able to shuffle or switch
421 their associated Symbiodiniaceae genera (e.g. [63]), especially corals that have obligate
422 relationships with a particular Symbiodiniaceae genus. We want to reiterate here that, irrespective
423 of corals' temperature tolerance thresholds in the future, given the fact that we are facing
424 continuous changes in the global climate through carbon emissions, symbiont shuffling might not
425 be sufficient to withstand frequent and prolonged seawater temperature anomalies as it is not a
426 common trait in all coral species.
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428

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630 **Figures**
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632 Figure 1. Map showing the reciprocal transplant experiment locations in the Kenting National
633 Park, Taiwan. NPP-OL – Nuclear Power Plant Outlet, NPP-IL – Nuclear Power Plant Inlet, and
634 WLT – Wanlitung.
635
636 Figure 2. Seawater temperature (weekly average) trend (A) and DHW (B) at three locations: Red
637 line – NPP-OL, Black line – WLT, and Blue line – NPP-IL, in 2014 and 2015. Typhoons in 2015
638 are represented as arrows.
639
640 Figure 3. Symbiodiniaceae genera trends (A) in five native cores of *P. verweyi* during the 2014
641 transplantation experiment and (B) in 30 native cores of *P. verweyi* during the 2015 transplantation
642 experiment. Each colour block represents one core sampled from one individual colony. In the
643 case of NPP-IL, no samples were collected after July 2015. X = the cores were dead.
644
645 Figure 4. Symbiodiniaceae genera trends in bi-monthly sampling of tagged mother colonies at each
646 location in 2014-2016. Each colour block represents one core sampled from one individual colony.
647 In the case of NPP-IL, there were no samples after November 2015.
648
649 Figure 5. Bar plot of STRUCTURE. Bayesian clustering analysis for eight loci genotypes among
650 the NPP-OL and WLT *P. verweyi* populations. This bar plot assumed the number of population
651 K=2. 1,000,000 MCMC runs.
652

Characteristics of microsatellite loci

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

1   **Table 1**. Characteristics of eight microsatellite loci for 60 colonies of *P. verweyi* collected at NPP-
2   OL and Wanlitung.
3

locus	Primers sequences	repeats	Size of alleles	Tm (℃)	No. alleles	H_E
PV9	F:†CAACTTAAATGGTATCATCGTG R: GTGCCCTATTTTATGTGACAA	$(AG)_{26}$	140-168	50	9	0.85
PV19	F:†TAGTCAGTGGCATCTGAGAGT R: CTCATTTCCTCCTAAGCTTTC	$(TG)_{30}$	161-197	53	16	0.83
PV22	F:†TCACTTGCTATAACCTTCTCCT R:TCCACCTCTCCAACTAGTTATC	$(TG)_{12}$	140-164	50	10	0.86
PV56	F:†TTGACTCGTCAATCACCTATC R: GCTAGCACTGATCAAACGAT	$(TC)_{14}$	144-166	50	6	0.75
PV57	F:†ACAGACAGAGACAGACAGAACA R: CAGTTCACCTGTCCATTTG	$(TC)_{14}$	103-119	50	6	0.60
Plsi4.02	F:†ACAATTCGGATATGTAGC R:GTTTCTTTGGTTTGGTTTGTTCTC	$(AAAC)_{11}$	136-170	50	14	0.85
Plsi4.24	F:†TTATCTTGGTTCAGACAGACAG R:GTTTGACAACTCTAATGAAGGTCAG	$(ACAG)_{10}$	126-158	59	9	0.77
PD31	F:†GACAAGTAATGTGTAAATCGTTGTCC R:†CTGTTAGAGTATCATGTCCTGAAGC	$(CCAT)7$	156	56	7	0.54

5   † primer attached M13 (-21) tail (5'- TGT AAA ACG ACG GCC AGT -3') (18 bp)
6

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.
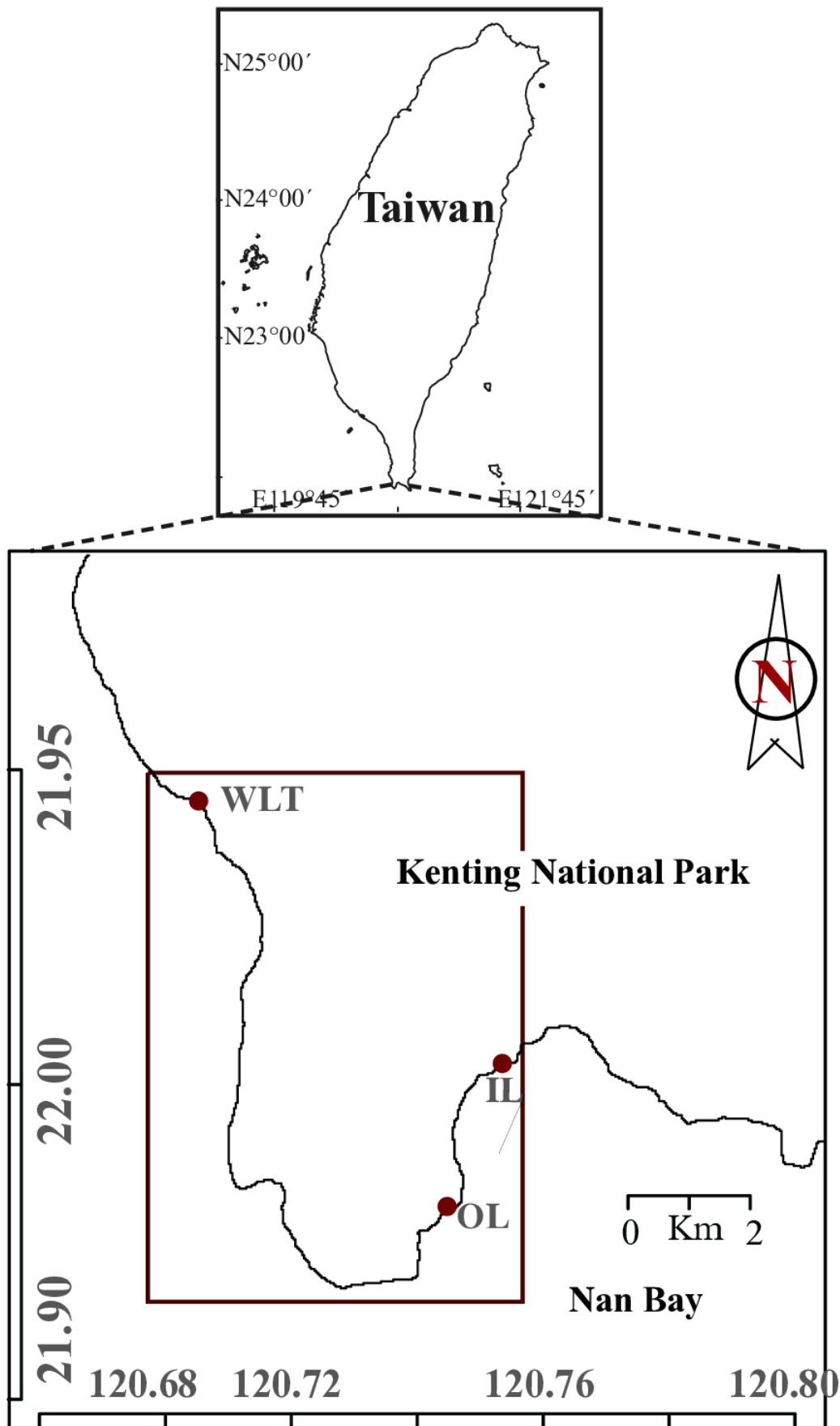
Figure 2

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
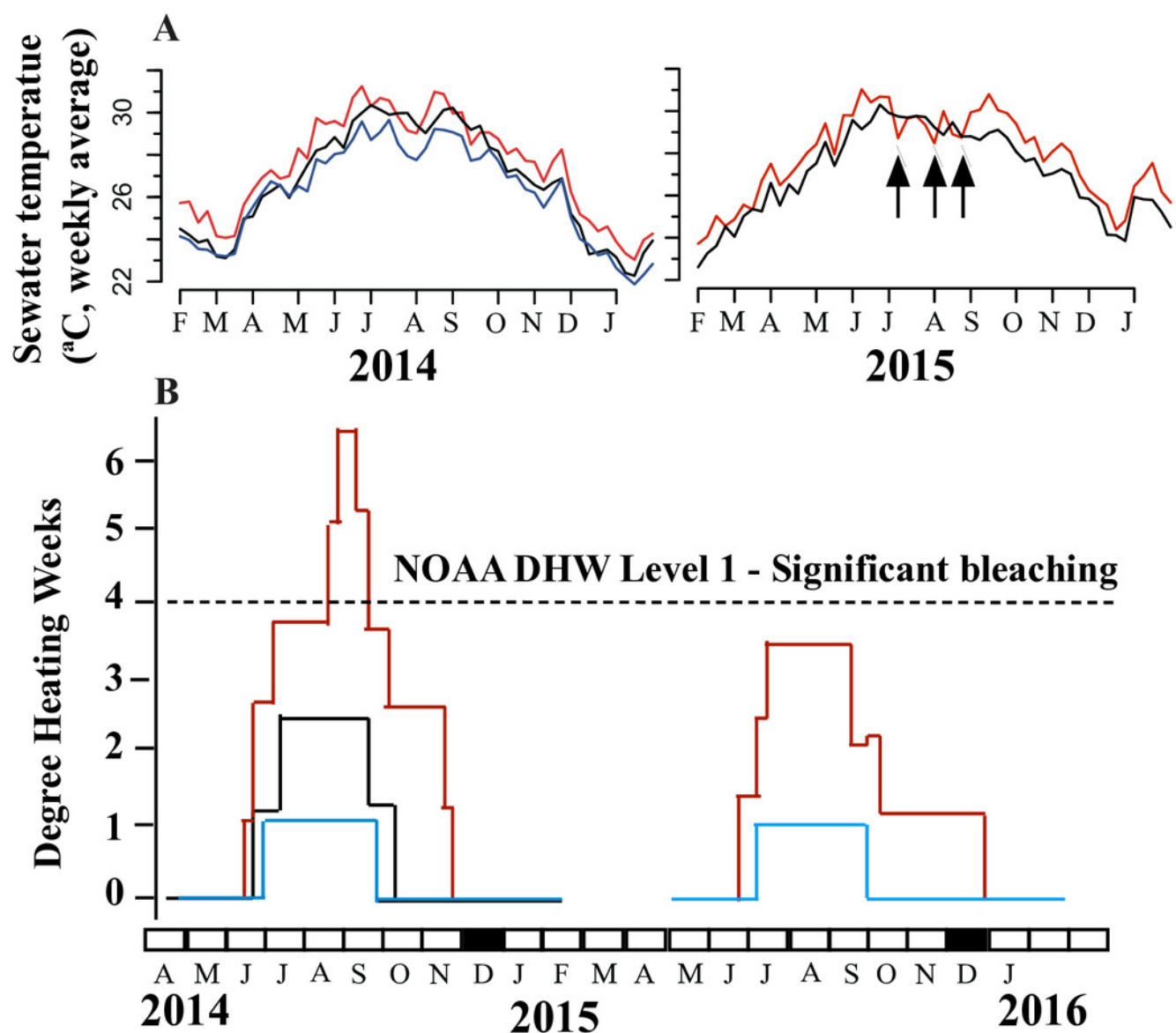
Figure 3

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.
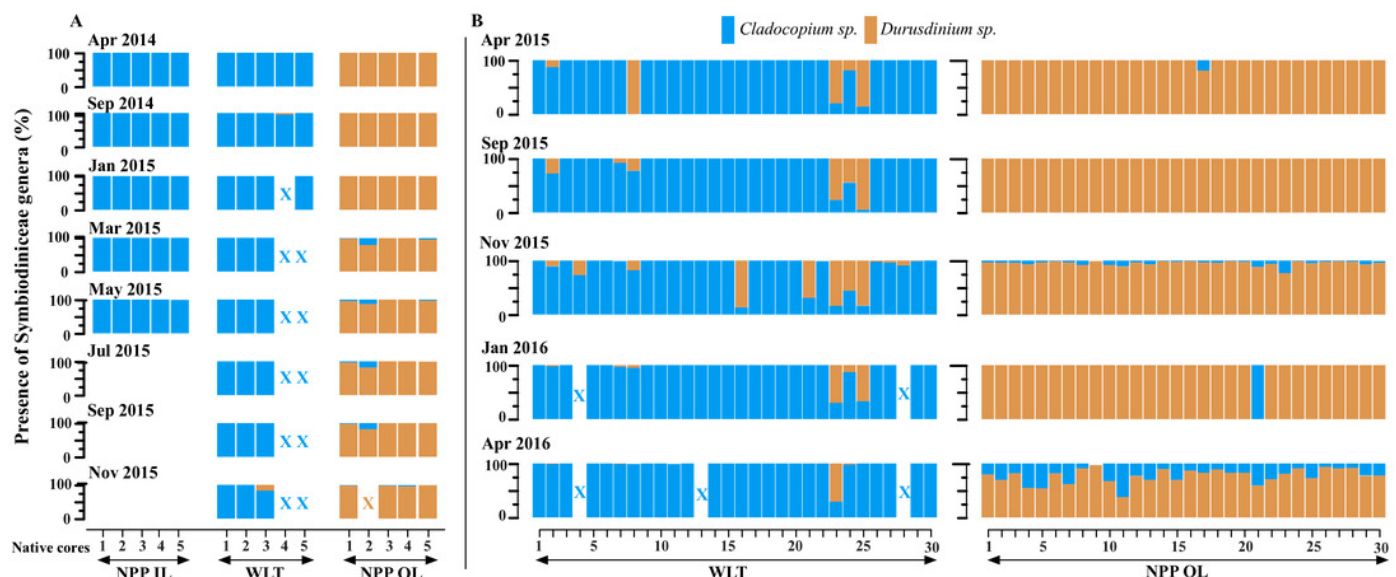
Figure 4

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.
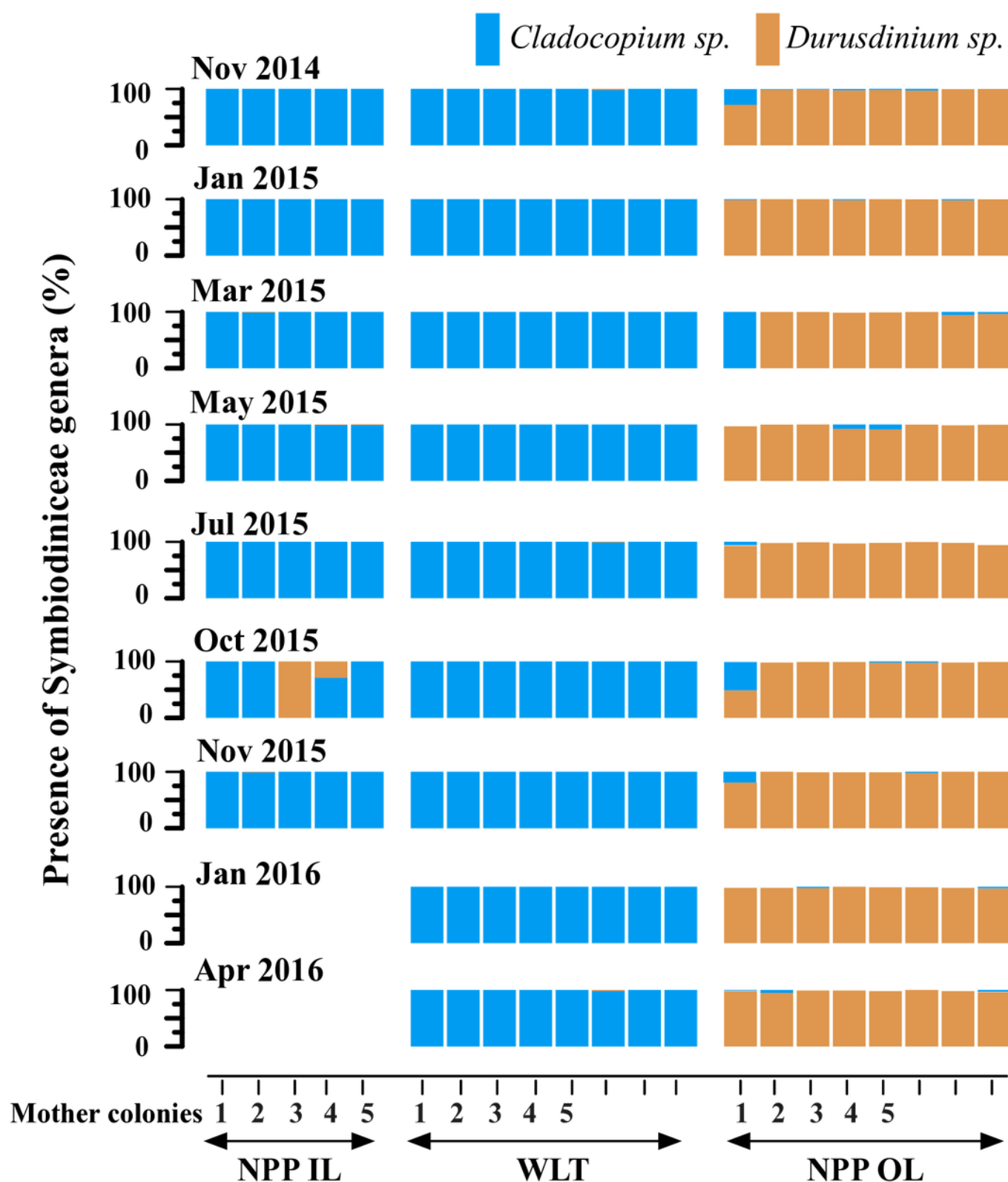
Figure 5

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.