1   Transgenerational inheritance of shuffled symbiont communities in the coral *Montipora*

2   *digitata*

3   Kate M. Quigley[1,2]*, Bette L. Willis[2], Carly D. Kenkel[1,3]

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5   [1]Australian Institute of Marine Science, PMB3, Townsville, QLD, Australia

6   [2]College of Science and Engineering, ARC Centre of Excellence for Coral Reef Studies,

7   James Cook University, Townsville, QLD, Australia

8   [3]Department of Biological Sciences, University of Southern California, Los Angeles CA,

9   USA

10   *Correspondence: Kate M. Quigley

11   Email: katemarie.quigley@my.jcu.edu.au

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17

18   **Abstract**

19   Adult organisms may "prime" their offspring for environmental change through a number of

20   genetic and non-genetic mechanisms, termed parental effects. Some coral species can alter

21   their thermal tolerance by shuffling the proportions of *Symbiodinium* types within their

22   endosymbiotic communities, but it is unclear if this plasticity can be transferred to offspring

23   in corals that have maternal symbiont transmission. We evaluated symbiont community

24   composition in tagged colonies of *Montipora digitata* from Orpheus Island, Australia, over

25   two successive annual spawning seasons, the second of which overlapped with the 2016 mass

26   coral bleaching event on the Great Barrier Reef. We applied amplicon sequencing of the ITS2

27   locus to samples of four families (parent colonies and 10-12 eggs per family) to characterize

28   their potential for symbiont shuffling and to determine if shuffled abundances were preserved

29   in gametes. Symbiont cell densities and photochemical efficiencies of the symbionts'

30   photosystem II differed significantly among adults in 2016, suggesting differential responses

31   to increased temperatures. Although abundances of the dominant symbiont haplotype, a

32   representative of clade C15, did not differ among families or over time, low-abundance

33   ("background") ITS2 types differed more among years (2015 vs. 2016) than between life

34   stages (parent vs. offspring). Results indicate that background symbiont shuffling can occur

35   in a canonically 'stable' symbiosis, and that such plastic changes to the symbiont community

36   are heritable. To our knowledge, this is the first evidence that shuffled *Symbiodinium*

37   communities can be inherited by early life-history stages and supports the hypothesis that

38   plastic changes in microbial communities may serve as a mechanism of rapid coral

39   acclimation to changing environmental conditions.

40

41   **Introduction**

42   Acclimatization to fluctuating environmental conditions through phenotypic plasticity can

43   promote the persistence of populations and may facilitate subsequent genetic rescue in an era

44   of global climate change [1,2]. In some cases, plasticity may also be associated with

45   transgenerational effects, whereby the performance of offspring is influenced by

46   environmental conditions experienced by the parents [3]. Inheritance of altered parental

47   phenotypes via transgenerational plasticity may further buffer populations by facilitating

48   acclimatization over generations [4,5].

49

50   In corals, the Adaptive Bleaching Hypothesis (ABH) [6] has been proposed as a

51   potential acclimatization mechanism that could protect adult corals from changing

52   environmental conditions through alteration of their endosymbiotic *Symbiodinium*

53   communities [7–10]. The proportional abundances of *Symbiodinium* types within adult coral

54   tissues can vary ("shuffling") to favour types that appear to be more tolerant of prevailing

55   environmental conditions [10–14]. Such plastic changes to the *Symbiodinium* community

56   have the potential to decrease the predicted frequency of bleaching, the breakdown of the

57   host-symbiont relationship as a result of stress, by 14% [15]. However, it is unclear whether

58   acclimatory changes to the *Symbiodinium* community are limited to the lifetime of an adult

59   coral or if shuffled communities are inherited across generations in coral species capable of

60   vertical symbiont transmission.

61

62   Vertically-transmitting coral species provision eggs or planula larvae with

63   *Symbiodinium* types predominantly from the maternal parent [16]. Genetic factors

64   influencing the overall composition of the *Symbiodinium* community in these early life stages

65   are inherited in at least three species [17,18]. As a heritable trait, genetic constraints (e.g. host

66   controlled immunity and recognition) may limit the capacity of vertical transmitters to

67   modify the diversity of their symbiont communities that are not found in species with

68   environmental acquisition [19]. Padilla-Gamiño *et al*. [20] examined *Symbiodinium*

69   communities in adults and eggs of the vertically-transmitting coral *Montipora capitata* across

70   years, and found that while community composition was predominantly faithfully

71   transmitted, the community did not change over time. In contrast, *Porites astreoides* planulae

72   differed in their symbiont communities compared to adults and juveniles [21]. However,

73   transgenerational inheritance of other traits, including symbiont switching, has been

74   suggested [22]. For example, larvae of the vertically-transmitting coral *Pocillopora*

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75   *damicornis* exhibited altered respiration rates when parents were exposed to elevated pCO_2

76   during the development of brooded larvae [23]. Given the major role symbionts play in coral

77   thermal tolerance [12], evaluations of the potential for increased ocean temperatures to alter

78   maternal transmission of *Symbiodinium* and of the capacity for rapid acclimation and

79   adaptation of offspring through transgenerational mechanisms are needed.

80

81   The importance of background (low-abundance) *Symbiodinium* types for the capacity

82   of corals to alter their thermal tolerance is becoming more widely recognised, in light of

83   growing evidence that the capacity to shuffle *Symbiodinium* usually involves a change in the

84   abundance of background species [14,24–26]. *Symbiodinium* from clade D, the taxa most

85   often reported as instrumental in post-bleaching recovery in corals, normally exist at low

86   levels in coral hosts, even as low as 100 D cells to 10,000 host cells per cm^2 [27,28].

87   Recently, Bay et al. (2016) found that some colonies of *Acropora millepora* from a naturally

88   cooler population with a ratio of at least 3:1000 D:C type symbionts were able to shuffle to a

89   D-dominated community during stress, and subsequently exhibited increased survival and

90   recovery. Background symbiont types are also known to be functionally important in other

91   host-microbe associations. For example, rare members of the bacterial community play

92   disproportionally important roles in nitrogen cycling in their hosts (e.g. *Desulfosporosinus* in

93   peat soil [29]). Therefore, changes in the relative abundances of background *Symbiodinium*

94   types have the potential to change the host phenotype, thereby contributing to its stress

95   tolerance and recovery [24,25].

96   We took advantage of a natural thermal stress event, which caused severe bleaching in

97   corals on the Great Barrier Reef (GBR) in 2016 [30], to evaluate the potential for

98   transgenerational inheritance of shuffled symbiont communities in a vertically-transmitting

99   coral. Using amplicon sequencing of the ITS2 locus, we compared *Symbiodinium*

100   communities in a sample set comprised of four colonies of *Montipora digitata* and 10-12

101   eggs from each colony collected during the 2016 mass bleaching event to a replicate sample

102   set collected during the previous summer (2015; no thermal anomaly). Although this species

103   is typically dominated by C15-type symbionts, it has been shown to host background Clade D

104   and A types [17]. We evaluated the null hypothesis that symbiont communities in *M. digitata*

105   are stable in response to thermal stress and highly heritable ([17], H_0, figure 1A) and three

106   possible alternative hypotheses: H_1- symbiont community composition is stable, but not

107   highly heritable (no shuffling, no parental effects); H_2- symbiont community composition is

108   plastic, and highly heritable (shuffling and parental effects); H_3- symbiont community

109   composition is plastic, but not heritable (shuffling, no parental effects).

110

111   **Materials and methods**

112   **Coral spawning and sample collection**

113   Thirty-two colonies of *Montipora digitata* were collected from Hazard and Pioneer

114   Bays in the Palm Island group on the Great Barrier Reef (GBR) on the 30th of March and 1st

115   of April 2015. The same colonies were re-collected from Hazard Bay in April 2016 three

116   days before the full moon, with the exception of colonies 4, 5, 8, 15 and 25, which were

117   presumed dead (n_{2016} =27). In both years, colonies were placed in constant-flow 0.5 µM

118   (2015) or 0.25 µM (2016) filtered seawater in outdoor raceways at Orpheus Island Research

119   Station. Egg-sperm bundles were collected from nine colonies in 2015 (23rd April) and four

120   colonies in 2016 (24th April). Eggs were filtered through a 100 µm mesh and rinsed three

121   times to remove sperm and then individually preserved in 100% ethanol. Branches (n_{2015} = 1

122   branch/colony; n_{2016} = 3 branches/colony) were in 100% ethanol for genetic analysis of the

123   *Symbiodinium* communities.

124

Evaluating adult physiology during spawning in 2016 (El Niño year)

To determine the impacts of the El Niño-driven mass thermal stress event on *M. digitata* in 2016, three traits commonly used to evaluate holobiont health were measured: effective quantum yield of photosystem II (YII), colony coloration, and *Symbiodinium* cell density. YII was measured using Pulse Amplitude Modulated fluorometry (PAM) with a fibre optic cable (diving-PAM, Waltz). PAM measurements were taken at mid-day (~12:30) one day prior to spawning (22nd) and two days during the spawning period (23rd, 24th). Three measurements were taken per colony at each time point, with the instrument set at an Intensity of 12, Gain of 4 and F_0 between 1.5 - 3. YII represents the percent quanta used by photosystem II of the *Symbiodinium* cells and is an indication of photosystem functioning and stress during light reactions. To test for significant differences in YII due to colony identity or day (as categorical factors), linear mixed models were fit using the package 'nlme' in R [31]. Replicate fluorescence measurements per colony were set as a random effect and uniform compound symmetry correlation structure (CorCompSymm) with Day as input values were used to account for autocorrelation issues. Analysis of Deviance Type II tests from the 'car' package were used to determine overall main and interactive effects [32]. The 'glht' package was used to extract Tukey p-adjusted values for multiple comparisons from linear models to determine if YII values differed among colonies [33]. Linear models with the same structure were used to test for significant differences in YII values between spawning and non-spawning colonies. Assumptions of homogeneity of variance, linearity, normality and autocorrelation were checked and tests modified where appropriate.

The presence of bleaching at the time of collection was assessed by photographing each colony alongside the CoralWatch color chart [34] to calibrate light conditions for image analysis. Three replicate RGB measurements were taken across each branch in ImageJ,

150   resulting in continuous numerical values between 2.17 and 6, where values greater than 6

151   were designated as 6 [34]. The average of RGB color at each of the three randomly selected

152   points per image were calibrated using photo-specific linear equations determined from

153   analyses of the simultaneously photographed CoralWatch color chart. A negative binomial

154   model (glmer function from 'lme4') was used to assess if mean bleaching status varied

155   significantly with colony identity (colony = fixed effect, replicate color chart measures per

156   colony = random effect). No deviations in homogeneity of variance, zero-inflation or over

157   dispersion were detected. Likelihood ratio tests (LRT) showed that there was no significant

158   effect of spawning status (spawning or not) or its interaction with dam identity on bleaching

159   status (both Pr (>Chisq) = 1), thus these terms were dropped. LRT and effect sizes were used

160   to assess the overall effect of colony identity on bleaching status (if the means differed

161   significantly across colonies) by comparing the full model to the null model without colony

162   identity using p-values calculated from a chi-squared distribution. *Symbiodinium* density was

163   determined from spawning colonies using a Neubauer Hemocytometer (Optik Labor, UK).

164   Triplicate cell counts were performed after nubbins were tissue blasted and standardized to

165   the surface area of each nubbin. Surface areas were calculated using the wax dipping method,

166   using 11 reference standard cylinders [35].

167

168   *Symbiodinium community composition analysis*

169   *Symbiodinium* communities in adults and eggs in both 2015 and 2016 were genotyped using

170   paired-end Illumina Miseq amplicon sequencing (2 x 250bp) of the ITS2 locus [36] at the

171   Genome Sequencing and Analysis Facility at the University of Texas at Austin (USA).

172   Samples from all spawning adults (n_{2015} = 9 branches, 1 branch per coral; n_{2016} = 12 branches,

173   3 branches from each of 4 corals) and 10-12 eggs from each colony were sequenced in two

174   independent runs (corresponding to 2015 versus 2016 samples).

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175

176   Raw fastq reads were pre-filtered by removing any pairs that contained Illumina sequencing

177   adapters (reads with an exact match of at least 12bp) or did not begin with the ITS2 amplicon

178   primer sequence (reads lacking an exact match of at least 10bp), using BBDuk from the

179   BBMap package version 37.75 (http://sourceforge.net/projects/bbmap/). ITS2 sequence

180   variants were then inferred using the DADA2 pipeline [37] in R (v 3.4.1)[38]. The following

181   analysis was completed twice, once for the full dataset, and once for a dataset in which the

182   2016 reads were randomly subsampled using seqtk (https://github.com/lh3/seqtk) to the mean

183   abundance for the 2015 dataset post-quality filtering (7,000 PE reads per sample). Briefly,

184   filtered fastq files were imported into R and read quality profiles were visually inspected.

185   Reads were then filtered further, removing reads exhibiting matches to the phiX genome,

186   reads with uncharacterized bases or reads with more than one expected error [37]. For reads

187   passing these filters, ITS2 amplicon primers were trimmed prior to variant analysis [37].

188   Error rate models for forward and reverse reads were run until convergence, and estimated

189   rates were visually inspected to assess fit with expected error rates. Sequence variants were

190   inferred from the entire sequencing dataset using the default options for the "dada" command,

191   which accounts for substitution and indel errors based on learned models, but the

192   "BAND_SIZE" flag was set to 32 as is recommended for ITS data [37]. Inferred variants

193   were further culled for length (294-304bp accepted) to remove potential products of non-

194   specific priming, and chimeric sequences were removed. These high confidence sequence

195   variants were taxonomically classified through a blast search against the GeoSymbio ITS2

196   database [39], and the best match was recorded. In cases where variants matched equally well

197   to multiple references, all top hits were reported.

198

199　The MCMC.OTU package [40] was used to remove sample outliers with low counts overall

200　(z-score <-2.5) and sequence variants that appeared in less than three unique samples prior to

201　statistical analysis. Only the subset of families for which spawning was captured in the two

202　consecutive years was retained in order to evaluate the impact of life stage and year on

203　symbiont community composition.

204

205　A principal coordinates analysis (PCoA) was performed by computing Manhattan distances

206　of log-transformed normalized variant counts.　The resulting plots were visualized using the

207　Phyloseq package [41] to examine the effects of the factors family, year and life stage on

208　*Symbiodinium* community composition. The DESeq package [42] was used to construct a

209　series of generalized linear models to evaluate differences in the abundance of sequence

210　variants with respect to life stage (adult/egg) and year (2015/2016), including family (7, 9,

211　11, 24) as a blocking factor. Models were run for 30 iterations and, for models that did not

212　converge, p-values were converted to NAs prior to applying a multiple test correction [43].

213　The Phyloseq package was used to plot results.

214

215　To assess the relationship between shuffling and bleaching, the composition of the

216　*Symbiodinium* community was converted to a single quantitative metric (full description and

217　methods in [17]) and correlated to the bleaching score. To facilitate comparisons between the

218　two years, only samples collected from the centre of each colony in 2016 were used, in order

219　to correspond to samples collected similarly in 2015.

220

221　Sequence variants of interest were aligned using Clustal Omega [44], as implemented on the

222　EMBL-EBI web server (https://www.ebi.ac.uk/Tools/msa/clustalo/) and haplotype networks

223　were visualized using the plot functions from the Pegas package [45]. Phylogenetic

224   relationships among significant clade C-type OTUs were determined using the Phangorn

225   package [46]. The Hasegawa-Kishino-Yano model of nucleotide substitution including

226   invariant sites [47] was identified as the best fit model based on AIC, and was used to infer

227   relationships among variants using 100 bootstrap replicates.

228

229   **Results**

230   *Montipora digitata* **physiology during spawning in a severe bleaching year (2016)**

231   Twelve of the experimental colonies showed variable signs of bleaching in 2016, as indicated

232   by the bleaching status metric ranging from 3.08 ± 0.5 (colony 16) to 5.84 ± 0.2 (colony 10).

233   Thirteen colonies did not exhibit any signs of bleaching or loss of pigmentation (bleaching

234   status = 6) (figure 2A). Regardless of this variability in bleaching status across colonies, there

235   was no significant relationship between bleaching status and colony identity, spawning status

236   of the colony, or an interaction between the two (identity, spawning status, identity*spawning

237   status: all NB LRT Pr(>Chisq) > 0.9996). *Symbiodinium* density varied significantly among

238   the four colonies that spawned (LRT, df = 6, p = 0.0097) (figure 2 inset); with three

239   spawning colonies exhibiting either no or light paling (5.8 ± 0.2) and colony 7 only moderate

240   paling (4.8 ± 0.5).

241   Overall, values for the effective quantum yield of photosystem II (YII) ranged from $0.699 \pm$

242   0.001 (colony n2) down to 0.618 ± 0.009 (colony 21). In general, YII values varied

243   significantly by day, increasing from an average of 0.67 ± 0.002 to 0.69 ± 0.004 in the three

244   days preceding and during spawning (n=25 colonies with three replicates measured per day);

245   Likelihood Ratio Test-LRT, df = 2, p = 1.5e-07, figure 2B). Average YII measures also

246   varied significantly among dams (LRT, df = 24, p = 2.2e-16), but in a consistent manner

247   through time (no significant interaction between dam identity and day: LRT, df = 48, p =

248   0.127). Spawning colonies had significantly lower YII values compared to non-spawning

249   colonies (0.66 ± 0.02 versus 0.68 ± 0.03; LME, df = 221, p = 1e-04).

250

251   ***Symbiodinium* community composition**

252   On average, more reads per sample were obtained for the 2016 sample set than for the 2015

253   sample set (32,708 versus 14,020 2 x 250bp PE reads, respectively), and more reads were

254   retained for the 2016 dataset following quality filtering (78% vs 33%). This resulted in an

255   average of 5,138 reads per sample in 2015 and 26,566 reads per sample in 2016 being

256   available for identification of ITS2 sequence variants (~types).

257

258   A total of 34 high confidence types (those occurring in more than three unique samples) were

259   identified from a subset of 107 high-read abundance samples (z-score > -2.5), representing

260   the four families originating from colonies that spawned in both years (colonies/families 7, 9,

261   11 and 24). Of these 34 types, a single variant comprised 66% of the normalized read data

262   across samples; a blast search against the GeoSymbio database (Franklin et al. 2012)

263   identified it as a best match to *Symbiodinium* type C15. Additional background types, ranging

264   in abundance from 17% to <1% of the normalized read data across counts also exhibited best

265   matches to type C15 (26/34) or some derivative thereof (2/34 C15.h, 1/34 C15.6, 1/34 C15.8,

266   1/34 C15.9). The remaining three background types exhibited best matches to the C1

267   reference (comprising 0.05% of normalized reads) and two Clade D variants (best-matches to

268   D1 and D1a, comprising 0.04% and 0.014% of normalized reads, respectively).

269

270   Principal coordinates analysis revealed that year was the main factor differentiating symbiont

271   communities among samples (figure 3). This pattern could have been driven by the greater

272   quantity of read data generated for the 2016 dataset. Therefore, the analysis was repeated on a

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273   dataset in which high coverage samples from 2016 were randomly subsampled to 7,000 PE

274   reads to mimic a reduced sequencing effort. The same major sequence variants were

275   recovered and year remained the main factor differentiating samples (figure S1,2).

276

277   A series of linear mixed model analyses further supported the dominant effect of year, with

278   twelve of these variants found to be significantly differentially abundant by year, life-stage or

279   the interaction of the two (figure 1B, 4). Only a single ITS2 sequence variant (sq2) was

280   identified as being significantly differentially abundant between life-stages alone. Sq2, which

281   comprised 17% of the read data across samples, was 1.25-fold more abundant in eggs than

282   adults ($P_{adj} < 0.05$, figure 4C). Sq4 (5% of reads) was also more abundant in eggs than adults,

283   by 1.27-fold ($P_{adj} < 0.05$), but showed an additional effect of sampling year, being 1.35-fold

284   more abundant in 2016 than 2015 ($P_{adj} < 0.05$, figure 4D). Both sq2 and sq4 exhibited a best

285   match to the GeoSymbio Clade C15 reference (figure 4A), and their abundances were

286   marginally positively correlated with one another across samples (R = 0.29). However, they

287   both exhibited strong negative or neutral relationships with the other C15-type variants,

288   suggesting that in spite of sequence similarity, they may not all be intracellular ITS2-type

289   variants (figure S3).

290

291   Abundances of ten sequence variants differed between sampling years, including sq4. Eight

292   sequence variants, all C-15 types, comprising 6.6% of total reads, were more abundant in

293   2016 (bleaching year) than in 2015 ($P_{adj} < 0.05$, figure 4B, D, F-M). Although the abundance

294   of sq8 increased across sampling years, increases were more pronounced in adults ($P_{year \times life}$

295    $_{stage} < 0.05$; figure 4F). In addition, abundances of sq3, sq8, sq10, sq11 and sq20 were

296   generally strongly positively correlated across samples, suggesting that they may represent a

297   cluster of intracellular variants. The largely independent positive relationship found between

298   sq18 and sq21 (R = 0.72) may indicate a separate intracellular type grouping (figure S3). The

299   tenth, a D1-type comprising only 0.04% of the total read data, was significantly less abundant

300   in 2016, by 5.25-fold on average, although this pattern was primarily driven by decreased

301   abundance in eggs and a concomitant tendency for increased abundance in adults ($P_{adj} =$

302   0.014; figure 4N). Only one variant, sq5, showed a pattern whereby abundance increased in

303   adults across sampling years but decreased in eggs, such that abundances in the two life

304   stages converged in 2016 ($P_{adj:year \times life\ stage} < 0.05$, figure 4E).

305

306   ***Physiological changes associated with shuffling***

307   Colonies that experienced no bleaching (11, 24) exhibited the greatest relative change in their

308   *Symbiodinium* communities (shuffling score), whereas *Symbiodinium* communities in

309   colonies that bleached (7, 9, bleaching scores 4.8-5.8) remained comparatively constant

310   (figure 5A). Although the sample size was small (n = 4 colonies), a moderate correlation

311   between community change and bleaching status was detected ($R^2 = 0.51$, figure 5B).

312

313   **Discussion**

314   This study investigated whether changes in the abundance of *Symbiodinium* types

315   within the endosymbiotic communities of corals in response to a bleaching event are heritable

316   in a coral species capable of vertically-transmitting symbionts to its offspring. Although

317   *Symbiodinium* communities in both adults and eggs of the coral *Montipora digitata* were

318   dominated by one, temporally-stable C15 type (66% of read data), variation in the

319   background or low-abundance *Symbiodinium* community occurred across years. Of the 34

320   sequence variants identified with high confidence, ten showed significant differences in

321   abundance between a bleaching and non-bleaching year, differences that were consistent in

322   adults and their corresponding eggs, supporting transgenerational inheritance of

13

323   *Symbiodinium* communities that had been shuffled in parental colonies in response to thermal

324   stress (figure 1, H_2). In contrast, the abundances of four sequence variants differed among life

325   stages, but patterns were stable across years (H_1). In two cases, the abundances of sequence

326   variants in eggs changed across years, but abundances differed independently from

327   communities in adults (H_3). Overall, the most common pattern in background types was

328   broad mirroring of shuffled adult communities in eggs of *M. digitata.* Our results suggest

329   that not only is the symbiont community composition plastic in adults of *M. digitata*, but also

330   that this plasticity is heritable.

331

332   The importance of shuffling background symbiont types to the health of corals is only

333   beginning to be understood. Although some adult corals can shuffle the abundances of

334   *Symbiodinium* types within their endosymbiotic communities in response to temperature

335   changes, thereby increasing the likelihood of survival [48–50], the implications of

336   transgenerational inheritance of these shuffled communities is completely unexplored. In

337   other maternally-inherited symbioses (predominantly bacterial), transmission efficiency of

338   symbionts is influenced by changes in temperature [51,52]. Shuffling and switching of

339   symbionts (secondary acquisition and replacement, respectively) are both prevalent in insects

340   [53], and the acquisition of particular symbionts during temperature stress have been shown

341   to provide protective benefits to the holobiont [54]. Furthermore, symbiont-mediated

342   transgenerational effects have been reported to play a role in immune priming in insects [55]

343   and may be common in plants [56]. Finally, variation in *Symbiodinium* community

344   composition in coral early life-history stages may serve no functional role in vertical

345   transmitters under non-bleaching conditions (e.g. growth, [21], but remains untested under

346   thermal stress. To our knowledge, we present the first evidence that shuffled proportions of

347 symbiont communities in adult corals are largely preserved in a species which transmits

348 *Symbiodinium* to its eggs.

349

350   Building on previous evidence of the heritability of symbiont transmission in *M.*

351 *digitata* [17], our results expand current understanding of the flexibility of the *Symbiodinium*

352 mutualism in a vertical transmitter by showing that these species may have greater flexibility

353 to vary their symbiont communities during times of stress than was previously thought. Early

354 longitudinal studies examining *Symbiodinium* community composition in vertically-

355 transmitting corals in response to naturally occurring thermal stress events led to the general

356 conclusion that communities were highly stable [57,58] although variation in communities

357 across temperature gradients has been observed [59]. Even when some shuffling was

358 observed among adult colonies, communities eventually reverted to their pre-bleaching

359 abundances following a recovery period [13,60,61], suggesting that plasticity was temporally

360 limited, although the length of this recovery period might span years (see [13,24]). Compared

361 to *M. digitata*, congeneric adult colonies of *M. capitata* exhibited no interannual changes of

362 symbiont community composition within individual adult corals or their gametes over time

363 [20]. Although the prevalence of shuffling as an environmental response mechanism is still

364 debated, inter and intraspecific variation has been reported in multiple studies [26,62,63].

365 Hence, the stability in *M. capitata* can potentially be attributed to the lack of genetic or

366 environmental variation (i.e. non-bleaching conditions insufficient to elicit shuffling) or

367 shallow sequencing depth. Thresholds for shuffling responses may therefore only be brought

368 on by extremely high or fast changes in environmental gradients (e.g. temperature, wave

369 exposure, turbidity; [64]. Regardless of the ultimate temporal persistence of such changes in

370 adult corals, we show here that this altered symbiont community can be transgenerationally

371 inherited, although further work is needed to determine if shuffling thresholds for heritable

372 parental effects are similar to those proposed for adults [26].

373

374   Patterns in the changing abundances of background *Symbiodinium* types in response

375 to thermal stress found here for *M. digitata* highlight the complexity in the physiological

376 properties conferred by endosymbiotic *Symbiodinium* on their coral hosts. In horizontal

377 transmitters, the primary pattern described in response to thermal stress so far has been

378 shuffling to increase the abundance of *Symbiodinium* types belonging to clade D (D1 or D1a)

379 during bleaching, followed by a decrease in these types once bleaching conditions subside

380 [11,14,26]. Indeed, shifts to a D-dominated community in adult colonies of *A. millepora* were

381 found to increase thermal tolerance by ~1-1.5°C [12]. While we observed a trend for D1 to

382 increase in adult corals, consistent with the increase in thermal stress across years, this

383 sequence variant comprised less than 0.05% of the overall community. Similarly, nine

384 sequence variants that were significantly more abundant during the 2016 bleaching year in

385 both adults and their eggs, exhibited a best match to a C15 reference type and comprised 12%

386 of the overall community. It has been shown that populations of *Symbiodinium* with the same

387 ITS2-type can exhibit different thermal tolerances, and in symbiosis can also differentially

388 impact physiological limits of the holobiont [8,65]. Correspondingly, the response of *M.*

389 *digitata* colonies to thermal stress by shuffling to increase the abundance of these different

390 strains of C15 may provide different physiological costs and benefits to their *M. digitata*

391 hosts which may underpin the differences in abundance. Furthermore, differences in the

392 abundances of two sequence variants, a C15 and a D1-type, which increased in adults but did

393 not show similar patterns in eggs, may reflect differences in costs and benefits conferred to

394 hosts between life-stages. Prior work in horizontally-transmitting species has suggested that

395 D1 may not always have a thermal protective function in the early-life stages of corals or

16

396   across species [66]. Finally, although read depth was significantly lower for 2015 samples

397   due to the two years being processed on independent sequencing runs, directional changes in

398   abundance and random subsampling to mimic reduced sampling effort both suggest that read

399   depth did not alter the main results. Additional information is therefore needed to elucidate

400   the roles that C15 and D1 might have in the *M. digitata-Symbiodinium* partnership.

401

402   The potential importance of numerically rare or background symbionts for the coral-

403   algal symbiosis has long been appreciated [67], but appropriate methods for detecting the rare

404   biosphere have only recently become available [24,68,69]. Consequently, the physiological

405   implications of such shifts are only beginning to be explored. Whilst some background

406   symbionts appear to have no functional role [70], recent network analyses of 46 coral genera

407   provide evidence of the importance of cryptic *Symbiodinium* communities, in which rare

408   symbionts appear to significantly contribute to symbiosis stability [71]. Similarly, the rare

409   bacterial biosphere is important to holobiont physiology for a range of both plant and animal

410   symbioses [29] and may act to acclimatize the holobiont to new conditions, as postulated for

411   corals [11,72]. Our finding that adult colonies that exhibited the largest shifts in

412   *Symbiodinium* communities also experienced the lowest levels of bleaching suggests that

413   shuffling symbionts contributes to buffering host physiology under environmental stress. We

414   also found small but significant differences in photochemical efficiency between spawning

415   and non-spawning colonies, although it is unclear whether the magnitude of these differences

416   are biologically significant. Previous research has presented a link between coral ability to

417   shuffle, buffering of photophysiological performance and spawning success when exposed to

418   cold stress [8]. Lower YII values potentially represent smaller deviations away from

419   photophysiological norms, linked to spawning success. Further work on the long-term

420   physiological and fitness consequences of this heritable plasticity would help to evaluate the

421   potential for this mechanism to facilitate rapid coral acclimation to changing environmental

422   conditions.

423

Data Availability:

425   All raw sequencing data has been deposited in the NCBI Sequence Read Archive under

426   Accession number SRPXXX.

427

Conflict of Interest Statement

429   The authors declare no conflict of interest.

430

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434

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437   *digitata* from Orpheus Island were collected under Great Barrier Reef Marine Park Authority

438   permit G10/33312.1 to BLW.

439

Authors and Contributors

441   KQ and CK conceived and designed the experiments, performed the laboratory work, data

442   analysis and figure preparation and wrote the manuscript. BW provided technical expertise,

443   provided reagents and critically revised the draft and approved the final version and are in

444   agreement to be accountable for all aspects of the work.

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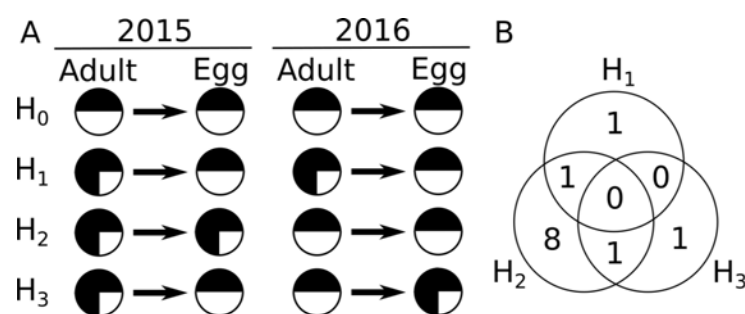638   *Bioscience* **43**, 320–326.

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641 **Figure 1.** (A) Schematic of hypotheses for potential parental effects and/or shuffling across

642 sampling years. (B) Venn diagram showing the number of significantly differentially

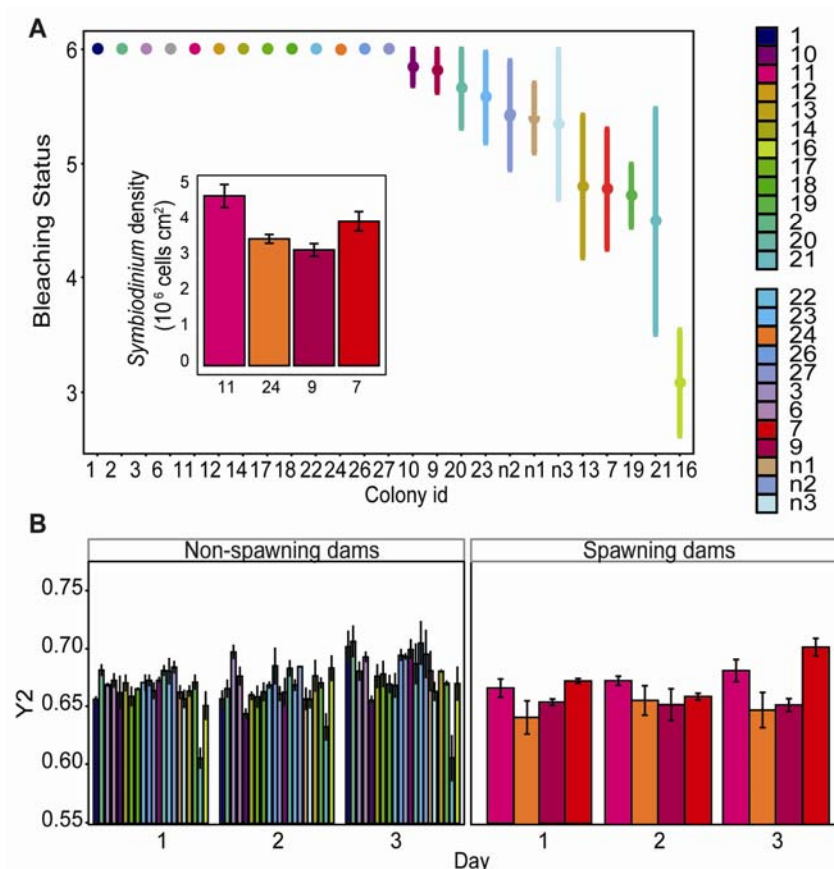643 abundant ($P_{adj} < 0.05$) sequence variants by hypothesis.

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647 **Figure 2.** *Montipora digitata* physiological measures during bleaching. (A) Bleaching scores

648 per colony during spawning 2016 (inset: average ± SE *Symbiodinium* density for spawning

649   colonies). (B) Effective quantum yield of photosystem II (YII) during the spawning period

650   for non-spawning and spawning colonies.
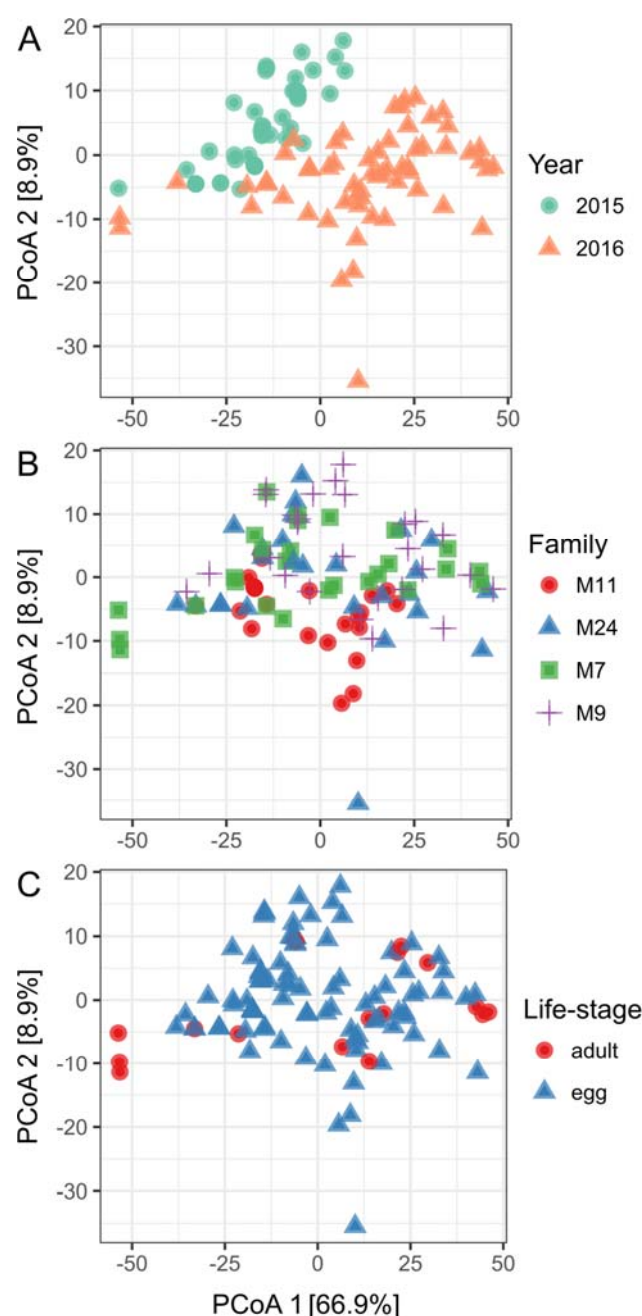
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Figure 3. Principal coordinates analysis (PCoA) of log-transformed Manhattan distances among normalized variant counts colored by (A) Sampling year, (B) Family and (C) Life-stage.
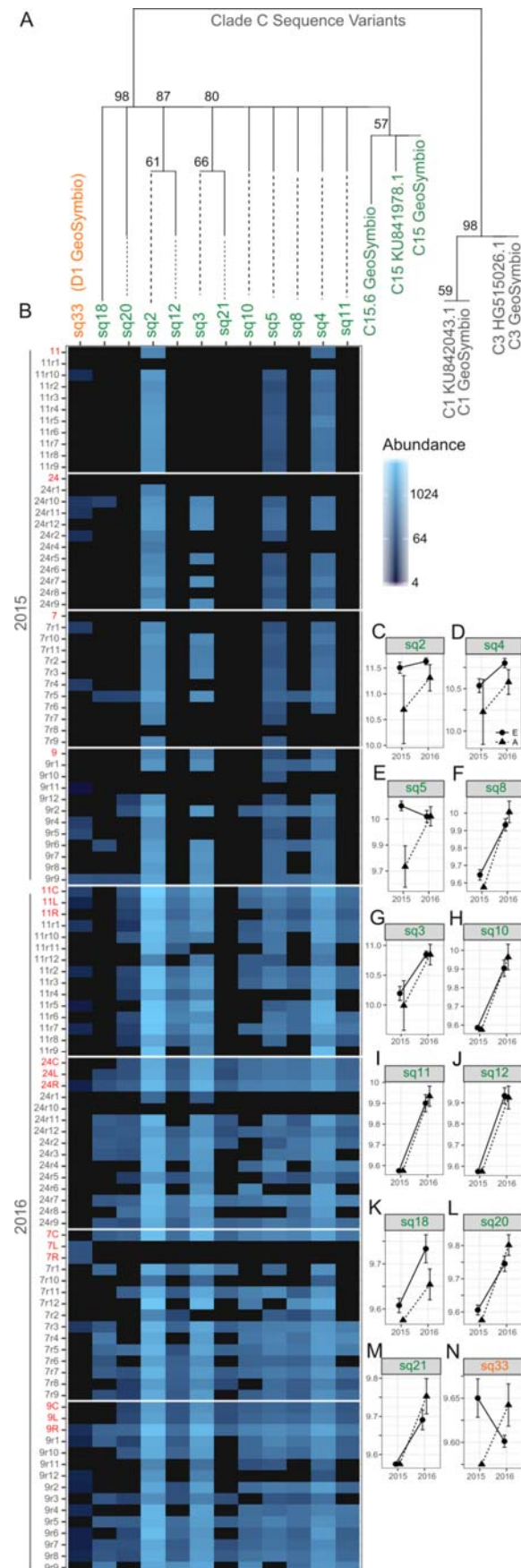
664   **Figure 4.** (A) Phylogenetic relationships among significantly differentially abundant (P_{adj} <

665   0.05) clade C-type OTUs. Reference types are either derived from the GeoSymbio database

666   or from NCBI's nr database. (B) Heatmap of showing read abundance by sequence variants

667   and samples, grouped by family and ordered by year and life-stage (adult samples are colored

668   red). (C-N) Mean variant abundance ± SEM for statistically significant factors: (C, D) P_{life-}

669     $_{stage}$<0.05, E=eggs, A=adults; (E,F) $P_{interaction}$<0.05; (D,F-N) P_{year}<0.05.
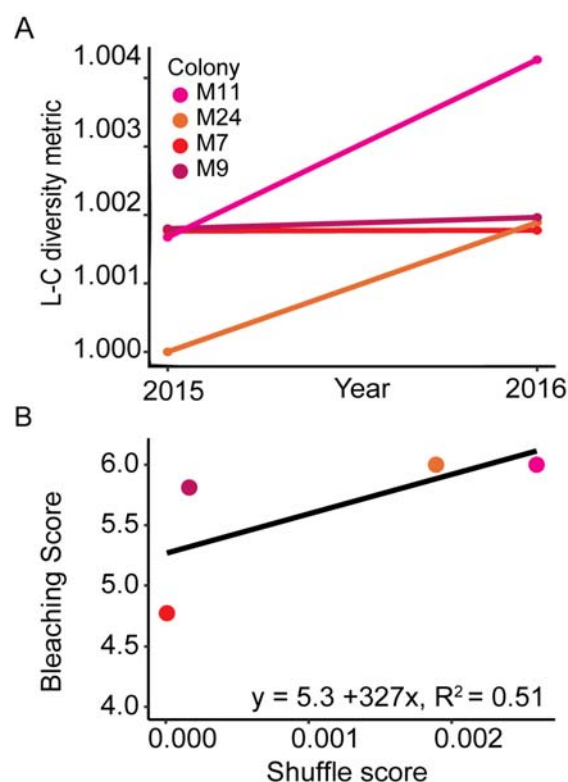
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673   **Figure 5.** (A) *Symbiodinium* community diversity metric in *M. digitata* during non-bleaching

674   (2015) and bleaching (2016) years for each of the four spawning colonies. (B) Relationship

675   between colony bleaching scores and the magnitude of shuffling in each spawning colony.

676   Low shuffling scores are indicative of communities that did not change between 2015 and

677   2016, whereas larger shuffling scores are indicative of *Symbiodinium* communities that did

678   change.