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Diversity of cryptic *Symbiodinium* clades in common coral genera from Moorea, French Polynesia: faithfulness does not exclude flexibility

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The adaptative bleaching hypothesis (HAB) states that depending to the symbiotic flexibility of coral hosts, coral bleaching can lead to a change in their composition of associated *Symbiodinium* community and contribute to the coral survival. In order to decipher this flexibility capacity the combined used of different molecular tools that privilege phylogeny and low-detection level of coral associated *Symbiodinium* are required. Here we used the highly sensitive qPCR technology to assess whether undescribed cryptic symbionts (in low abundance) are present in five common coral species of Moorea (French Polynesia), previously screened with conventional molecular methods. As usual, each coral species were characterized with a strong faithfulness to a particular clade(s), whatever the environment. But more unexpected, we showed that each of the five species harboured at least one additional *Symbiodinium* clade (among clades A-D), including the clade B, recorded for the first time as a coral symbiont in French Polynesia. Each of the clades A-D was detected at least once at a cryptic level that could play a role in the HAB proposed 'shuffling' mechanism. These results increased the census of the different coral-*Symbiodinium* patterns and revealed the current underestimation of the ability for corals to associate with a range of clades. Altogether our data highlight that corals have to deal with two opposite trade-offs: optimizing the symbiosis with faithful clade(s) and maintaining the ability to harbour different clades, making possible shifts of their *Symbiodinium* community face to environmental changes.

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ABSTRACT

The adaptative bleaching hypothesis (HAB) states that depending to the symbiotic flexibility of coral hosts, coral bleaching can lead to a change in their composition of associated *Symbiodinium* community and contribute to the coral survival. In order to decipher this flexibility capacity the combined used of different molecular tools that privilege phylogeny and low-detection level of coral associated *Symbiodinium* are required. Here we used the highly sensitive qPCR technology to assess whether undescribed cryptic symbionts (in low abundance) are present in five common coral species of Moorea (French Polynesia), previously screened with conventional molecular methods. As usual, each coral species were characterized with a strong faithfulness to a particular clade(s), whatever the environment. But more unexpected, we showed that each of the five species harboured at least one additional *Symbiodinium* clade (among clades A-D), including the clade B, recorded for the first time as a coral symbiont in French Polynesia. Each of the clades A-D was detected at least once at a cryptic level that could play a role in the HAB proposed '*shuffling'* mechanism. These results increased the census of the different coral-*Symbiodinium* patterns and revealed the current underestimation of the ability for corals to associate with a range of clades. Altogether our data highlight that corals have to deal with two opposite trade-offs: optimizing the symbiosis with faithful clade(s) and maintaining the ability to harbour different clades, making possible shifts of their *Symbiodinium* community face to environmental changes.

KEYWORDS:

Corals; *Symbiodinium*; clade B; French Polynesia; qPCR; flexibility; generalist; faithful clade

INTRODUCTION

The foundation of coral reefs is due to the symbiotic association between scleractinian corals and microalgae of the *Symbiodinium* genus. These microalgae are highly diverse, with a current identification of nine clades (A-I), each of them being partitioning into sub-clades based on genetic phylogenies (Pochon, Putnam & Gates, 2014). However, corals are most commonly associated with clades A-D (Baker, 2003), and in few cases with clades F and G (LaJeunesse et al., 2010; Putnam et al., 2012; Lee et al., 2016). The microalgae is assumed to provide ~~about~~ 95% of required energy for coral metabolic activities (Muscatine & Porter, 1977; Davy, Allemand & Weis, 2012), due in particular to their photosynthetic activity (*i.e.* carbon products). In return, the algae benefits from coral by receiving protected habitat from predation in the tissues, and a source of inorganic nutrients derived from host metabolism. However, this successful coral-algal association can ~~be broken because of~~ various stressors of both natural and anthropogenic origin (*i.e.* increasing seawater temperature, ocean acidification, sedimentation (Pandolfi et al., 2011), according to the resistance degree of both partners.

Coral species are described according to various biological and functional traits, such as: (i) morphology (e.g. branching, massive) (Yost et al., 2013), (ii) growth rates, (iii) reproduction (Baird, Guest & Willis, 2009), (iv) gamete fecundation (*i.e.* brooder or broadcast spawners (Baird, Guest & Willis, 2009), or (v) *Symbiodinium* acquisition (*i.e.* vertical or horizontal; Stat, Carter & Hoeghguldberg, 2006; Fabina et al., 2012). They are also characterized with different sensitivities to environmental conditions which are correlated to specific characteristics (e.g. morphology; van Woesik et al., 2011). For example, massive corals, such as *Porites*, are described to have higher resistance to environmental stressors (e.g. temperature anomalies; Penin, Vidal-Dupiol & Adjeroud, 2012). This is shown by lower mortality and/or bleaching rates of *Porites* genera, compared to the rates observed for branching corals such as *Acropora* and *Pocillopora* genera. In the same way, both *in situ* (e.g. Rowan et al., 1997; Baker, 2003; Berkelmans & Van Oppen, 2006; Sampayo et al., 2008) and *in vitro* physiological studies (e.g.

Banaszak, 2000; Kinzie et al., 2001; Hennige et al., 2009) suggested that *Symbiodinium* clades are characterized by specific physiological properties which are responsible for their sensitivity to various environmental conditions.

Within the same coral colony, a spatial partitioning of the different clades has been observed, depending on the depth and/or on irradiance (Rowan et al., 1997). Moreover, a diversity of coral-*Symbiodinium* associations, including mono or multi-clades associations, have been described in different coral colonies from a same species or during coral ontogeny (Little, Van Oppen & Willis, 2004), and/or in various environmental conditions opposing 'normal' *vs.* 'stressful' (e.g. seawater temperature anomalies, Berkelmans & Van Oppen, 2006). *Symbiodinium* clade D has notably been identified predominantly in resistant coral colonies during and after massive bleaching events, and/or more generally in reefs exposed to local stressors (e.g. sedimentation, eutrophication; Van Oppen et al., 2001; Ulstrup & Van Oppen, 2003; LaJeunesse et al., 2010, 2014; Cooper et al., 2011). These observations highlighted the importance of the various coral-*Symbiodinium* clade associations in thermo-tolerance capacity (Berkelmans & Van Oppen, 2006; Stat, Carter & Hoeghguldberg, 2006; LaJeunesse et al., 2009). Therefore it is currently suggested that coral flexibility for new *Symbiodinium* clade acquisitions may provide an ecological advantage in the context of environmental changes. This assumption, resulting in a range of various combinations of host-*Symbiodinium* associations, is the foundation of the 'Adaptive Bleaching Hypothesis' (ABH; Buddemeier & Fautin, 1993). The ABH asserts that there is potential for rapid 'adaptation' of corals facing stressful conditions by a dynamic modification of their *Symbiodinium* community composition either by i) acquisition of resistant *Symbiodinium* clades from free algae present in the environment, '*switching*', or ii) repopulation by cryptic pre-existing resistant *Symbiodinium* clades, '*shuffling*'. In the frame of this hypothesis, coral flexibility is of ~~upmost~~ importance and its study led to the characterization of two classes of coral host, the 'specialist' (associated to a specific *Symbiodinium* clade) and the 'generalist'

(associated to multiple *Symbiodinium* clades; Fabina et al., 2012; Putnam et al., 2012; Silverstein, Correa & Baker, 2012).

The development of molecular tools with highly sensitive detection capacities such as real time quantitative PCR (qPCR), which is up to 1000 times more sensitive than conventional methods (e.g. cloning, DGGEs, RFLP; Mieog et al., 2007), already suggested that corals may be more flexible (Mieog et al., 2007, 2009; Silverstein, Correa & Baker, 2012). In the present work, we used a qPCR approach to assess the *Symbiodinium* clade composition (from A to F). Each *Symbiodinium* clades were quantified within the five species of coral *Acropora cytherea*, *Acropora, pulchra*, *Pocillopora damicornis*, *Porites rus* and *Pavona cactus*, representing the four most common coral genera of Moorea island. These five coral species studied display different biological traits and were characterized with various resistance during local severe bleaching events of 2002 and 2007 (Penin, Vidal-Dupiol & Adjeroud, 2012). This study was designed to estimate the relative abundance of each clade within a host and therefore to distinguish between cryptic (*i.e.* a clade present in minority amongst a coral multi-clade pattern) and abundant clades. Altogether our data showed that all five coral species, 'specialists' or 'generalists', were associated to multiple *Symbiodinium* clades. However, despite this flexibility, each coral species displayed a major specific *Symbiodinium* clade association, which therefore led us to introduce the concept of faithful clade.

MATERIALS & METHODS

Choice of coral species

Five coral species of the fringing reef (0.5-2 m) of Moorea island (Fig. 1) in French Polynesia (17°30'9S, 149°50'9W) were chosen among the most commonly scleractinian coral genera from the Pacific: *Pocillopora (P. damicornis type β as described by Schmidt-Roach et al., 2014; Genbank reference xx-xx)*, *Acropora* (*A. cytherea* and *A. pulchra*), *Porites* (*P. rus*) and *Pavona*

(*P. cactus*). The differences in their morphologies, mode of *Symbiodinium* transmission and degree of sensitivity ~~degrees~~ to environmental conditions justified the choice of the species (Table 1**)**. *Acropora* is considered as the sentinel coral genus, being described with the higher sensitivity to environmental stressors (e.g. McClanahan et al., 2007; Penin et al., 2007; Penin, Vidal-Dupiol & Adjeroud, 2012). It was represented with two morphologically distinct species (branching *vs*. tabular). Conversely, the genus *Porites* was chosen for its high resistance to stress (e.g. Kayal et al., 2012; Penin, Vidal-Dupiol & Adjeroud, 2012), showing high partitioning in various habitats on the island (*i.e.* sedimentary bays). Finally, the two other genera, *Pocillopora* and *Pavona* were both considered to have intermediate degrees of sensitivity (Penin, Vidal-Dupiol & Adjeroud, 2012). All coral species were sampled during the dry season between August and October 2012 (n= 6-27; Table 1), with a bigger sampling effort on *P. damicornis*, *P. rus* and *A. cytherea*, ~~also assessed during a spatio-temporal survey (data not shown~~). Samplings were executed among five contrasted fringing reefs from the lagoon of Moorea island: Mahareapa (Ma) and Vaiare (Va) exposed to anthropogenic influence, and Teavaro (Te), Linareva (Li) and Tiahura (Ti) more isolated from human activities (Rouzé et al. 2015, Fig. 1).

DNA extraction

Small fragments of corals (0.5-1 cm^3), sampled at several locations of the top of each coral colony, were directly broken into a tube underwater and then immediately transferred at the surface in a new 1.5 mL centrifuge tube containing 80% ethanol. Until DNA extraction, all samples were stored at - 20°C. The ethanol was discarded and the sample gently rinsed with sterile freshwater to eliminate all trace of the mucus before extraction, in order to target only *Symbiodinium* present in the host tissues.

Total coral DNA (*i.e. Symbiodinium*, polyp and associated micro-organisms DNAs) was extracted using CTAB-based extraction protocol adapted from Mieog *et al. (Mieog et al., 2009)*. To

increase efficiency of DNA extraction, coral samples incubated in 600 µL extraction buffer CTAB 2% (2% CTAB, 1.4 M NaCl, 20 mM EDTA pH 8, 100 mM Tris-HCl pH 8 and 20 µg/mL proteinase K) were first exposed to 3 cryo-shock cycles (5 min in nitrogen liquid following by 10 min at ambient temperature), and then incubated at 60°C overnight while rotating. Next, the CTAB buffer was recovered and placed into new tube, in which was added 600 µL of chloroform/iso-amyl alcohol (24:1 vol/vol). The resulting solution was mixed thoroughly and centrifuged for 15 min at 12000 g (4°C). The aqueous phase was then transferred to a new tube and mixed with 600 µL ice cold isopropanol and incubated for 20 min at -20°C. After a new round of centrifugation, the supernatant was discarded and the pellet rinsed with 500 µL of 70% ethanol. After a last centrifugation of 10 min at 12000 g, the ethanol was removed and the DNA pellet air-dried before dilution in 100 µL sterile water (Sigma). All DNA samples were then stored at -40°C.

qPCR assay

Symbiodinium quantification

A real-time quantitative PCR assay (qPCR) on nuclear ribosomal DNA gene (rDNA) was used to quantify the proportion of symbiotic algae in corals. Firstly, specificity, sensitivity and efficiency of clade-specific primer sets (from A-F) on nuclear 28S rDNA region (Yamashita et al., 2011) was verified ~~from strain culture DNAs study~~ (*i.e.* strains from BURR Collection: clade A: CasskB8, B: Pe, C: Mp, D: A001, E: RT383 and F : Sin). Briefly, seven DNA extracts from ~~strain~~ culture belonging to clades A-F (e.g. one to two reference strains per clade) were mixed at the same concentration. A total of 70 ng of this DNA mix per PCR reaction was used to determine the specificity and sensitivity of each tested Yamashita' clade-specific primer (Yamashita et al., 2011). Efficiency of the qPCR reaction was then determined by tenfold dilutions of the DNA mix. Secondly, absolute quantification of each *Symbiodinium* clade (from A to F) was evaluated using

purified PCR products targeting the ribosomal 28S DNA region from each *Symbiodinium* clade culture DNA. PCR products were obtained with universal-clades primer set from Richter *et al. (2008)* for clade A-D and F (~580 bp), and from Wilcox (1998) for clade E (830 bp) and mixed together at the same concentration. Each *Symbiodinium* clade quantification using Yamashita's clade-specific primer was converted in 28S copy number from clade-specific standard curves obtained from serial 10-fold dilutions of the mix including the six universal clade 28S PCR products (Table S2), initially concentrated at 1 pg per qPCR reaction for each clade PCR products (equivalent to $1.73.10^6$ copy 28S for clade A-D and F, and $1.21.10^6$ copy 28S for clade E).

Polyp quantification

To compare *Symbiodinium* abundance between the different samples, the relative abundance of each clade within a host was required. Therefore, a polyp quantification was estimated to express a ratio of 28S copy number of *Symbiodinium per* copy number of polyp. Therefore, a region of the coral 18S rDNA was amplified (~100 bp) with universal coral primer set (univPolyp-18S F: 5'-ATCGATGAAGAACGCCAGCCA-3' and univPolyp-18SR: 5'-CAAGAGCGCCATTTGCGTTC-3'). These primers were designed with Primer 3 software from the 18S rDNA sequence alignment (276 sequences) of the 18 coral species among the most abundant genera found in French Polynesia such as *Porites*, *Pocillopora*, *Acropora and Povona* and of *Symbiodinium* clades, as negative control. Specificity and efficiency of the polyp primer set was tested from serial 10-fold dilutions (50 to 5.10^{-4} ng per qPCR reaction) of a mix of DNAs from ten coral species at the same concentration. For each coral DNA samples, Ct values were assigned to an arbitral value of polyp unit, estimating by the 18S copy number using the standard curve equation (Fig. S1b), in order to be further able to normalize the quantification of the *Symbiodinium* clades by a same amount of 18S rDNA gene targets (log+1 28S copy number of *Symbiodinium* on log+1 18S copy number of polyp). Calculation of *Symbiodinium*/host ratio (28S

copy number/ 18S copy number) allowed the comparison of the proportion of the different *Symbiodinium* clades in a coral DNA extract and/or between different coral DNA extracts.

All qPCR assays were conducted on a MX3000 Thermocycler (Stratagene) using SYBR-Green. Each reaction was performed, in a final volume of 25 µL containing: 12.5 µL of Brillant® SYBR Green Master Mix reagent, 2.5 µL of both reverse and forward primers diluted at the concentration of 4 µM, and 10 µL of DNA at various concentrations for standard curves analysis or at 1 ng.µL^{-1} for field samples analysis (NanoDrop® ND-1000 spectrophotometer). The following run protocol was performed: 1 cycle of pre-incubation of 10 min at 95°C; 40 cycles of amplification: 30s at 95°C, 1 min at 60°C or 64°C for the *Symbiodinium* and polyp respectively, and 1 min at 72°C; and a final step, for melting temperature curve analysis, of 1 min at 95°C, 30s at 60°C and 30s at 95°C. Each sample was analysed twice on the same plate, as one technical replicate, and averaged when the variation between both Ct values was not exceeding 1 (if not, samples re-process until $\Delta Ct \leq 1$). An interplate calibrator, tested in triplicate (one technical replicate), was added to set the threshold manually to calibrate and compare between different plates of coral DNA samples. Positive amplifications were taken into account only when both technical replicates produced Ct values between the estimated threshold ranges (*i.e.* sentivity: Ct limit of quantification; Table S1) after correction with interplate calibrator. In addition, all melting curve analyses ensured the specificity of the amplifications (Table S1). For new partnerships between *Symbiodinium* clade(s) and coral species we further purified the qPCR products (~100 bp) using QiaEx II Gel Extraction Kit (Qiagen GmbH, Hilden, Germany) and then sequenced in both directions by GATC Biotech (Cologne, Germany).

Statistical analysis

For each *Symbiodinium* clade, positively quantified in coral DNAs, the symbiont/host ratio datas (*i.e.* S/H ratio) were log+1 transformed for further analyses.

Percentage of specificity (S) of specific-clade primer sets was calculated according to the formula: $S= 1- \sum (100/2^{(Cti-Ctx)})$, where Cti and Ctx are Ct obtained from the specific-clade primer set and from others specific-clade primer sets, respectively. All the qPCR assays for validation of the method were tested on standard linear regression curves obtained from each primer set. Sensitivity of each clade specific qPCR assay was evaluated by comparing the Ct values obtained for each standard curves using a same 28S copy number in the reaction. Slopes, intercepts and Pearson correlation coefficient (R^2) were evaluated and compared by pairwise comparisons with Student's t-tests.

Discriminant analysis of principal components (DAPC) on S/H ratios, available for the 5 coral species, was performed (in R, package ade4) in order to characterize their preferential endosymbiotic assemblages. Therefore, the discrimination was applied through the coral species as factor.

RESULTS

Validation and optimization of qPCR assay

For all clade-specific primer sets, the specificity of each qPCR assay was greater than 98% and characterized by a unique melting temperature (Table S1). All clade-specific primers showed similar high efficiency with a correlation coefficient R^2 ranging from 95% to 101% whatever the tests performed either on DNA from *Symbiodinium* culture strains or purified PCR products, and non-significantly different linear regression slopes of standard curves between different clade-specific primers (Fig. S1). The threshold of 28S detection for each of the 6 tested clades was inferior to 200 copy numbers of 28S gene. The sensitivity of the clade specific primers, allowed two groups of primer sets to be distinguished. The intercepts pairwise comparisons (Student's t-test, $p<0.05$) between standard regression lines on 28S amplicons

231 showed earlier detection for primers specific to clades A, B, E and F (i = 16.36±0.39) when
232 compared with primer clade-specific to C and D (i = 19.83±0.27).
233 The relationship between Ct values and logarithmic plot from total DNA of corals (mix of
234 ten coral species DNA samples) yielded to good fit linear regression with an R^2 of 0.999 and an
235 efficiency of 101% (Fig. S1 and Table S1).

236 **Diversity and flexibility of 'dominant' vs. 'cryptic' *Symbiodinium* clades in corals**
237 The three *Symbiodinium* clades A, C and D (among the clades A-F tested) were detected
238 at least once in association with each of the five coral species studied, except for *P. cactus* which
239 was never found associated with clade A (Fig. 2). For some coral species these detections
240 revealed still undescribed clade partnerships for Moorea: C for both *Acropora* species *A. cytherea*
241 and *A. pulchra*, D for *P. cactus*, and A and D for *P. rus* (Table 2). The corresponding clade
242 sequences obtained on the 28S rDNA portion for these novel coral-*Symbiodinium* partnerships
243 revealed the presence of sub-clades: A13, C15, C1, C*x*, and D1 (Table 2). In addition, the first
244 record of *Symbiodinium* clade B as coral symbiont in French Polynesia was detected in *P.*
245 *damicornis* (n=2; Fig. 2), systematically in low proportions (<25%, Fig. 2). The unexpected
246 positive detection of clade B was ascertained through sequencing with the identification of sub-
247 clade: B1 (Genbank reference: xxx). Moreover two slight profiles in temperature melting curves
248 were obtained with specific clade C amplification for *P. rus*. Their sequences showed that each
249 profile corresponded to two distinct sub-clades: Cx (Tm~82.95°C; Genbank reference: xx) and
250 C15 (Tm~83.5°C; Genbank reference: xxx).
251 The occurrence of the four clades detected A-D led to fifteen possible theoretical patterns
252 among which nine have been observed, including assemblages of three clades together (Fig. 2):
253 ACD (*A. cytherea* and *A. pulchra*), BCD (*P. damicornis*) or ABC (*P. damicornis*). The six
254 missing possible *Symbiodinium* patterns systematically included clade B either as unique clade

(B) or as additional clade (BA, BC, BD, BAD and ABCD). The quantification of each clade through the ~~ratio S/H~~ led to an estimation of their relative proportion in the coral host (Fig. 2) and allowed us to classify them as 'dominant' (≥25%) *vs*. 'cryptic' clade(s) (<25%). *Symbiodinium* clade B, only detected in *P. damicornis*, was always characterized as 'cryptic' whatever the clade patterns and was systematically associated with at least clade C. All of the three other clades A, C and D have been observed at least once as cryptic clades, depending on the species and on the clade pattern. For example, the clade A was 'cryptic' in *P. rus* AC-pattern, while the clade D was 'cryptic' in *P. rus* CD-pattern. The clade C was observed as cryptic clade only once, in *P. damicornis* CD-pattern (Fig. 2).

Selective coral-*Symbiodinium* partnerships

The discriminant analysis of principal component (DAPC; Fig. 3) on the five coral species showed compositional differences among associated communities of *Symbiodinum* according to the clade identity and to their proportion into the host. The first axis (43.9% of total variance) of the DAPC opposed *Symbiodnium* communities characterized with clade D quantity (Pearson's corelation: $P < 0.001$, t=15.7) from communities composed with clade C (Pearson's correlation: $P < 0.001$, t=-21.5) and/or cladeB (Pearson's correlation: $P = 0.01$, t=-2.5). Clade D was strongly representative of corals *P. damicornis Symbiodinium* communities (100% of coral colonies sampling), nearly always appearing as unique clade (24/27=89%; Fig. 2). In contrast, *P. rus* (18/21=85.7%; Fig. 2) and *P. cactus* (6/7=85.7%; Fig. 2) colonies were nearly exclusively composed of mono-clade C communities. However, one *P. cactus* colony was also clade D associated (Fig. 2), underlying a larger variation range of associated symbiotic communities (wide size of discriminant ellipse, Fig. 3). The second axis (24.9% of total variance) of the DAPC differentiated *Symbiodinium* communities dominated with clade A (Pearson's correlation: $P < 0.001$, t=11.4) representative of both species from genus *Acropora*. These two species mainly

associated with multi-clade communitities (*A. cytherea*: 81% and *A. pulchra*: 67%) were distinguished by their second preferential clade in addition to the clade A (Figs. 2, 3): D for *A. cytherea* (AD and ACD patterns 11/16=68.8%) and C for *A. pulchra* (AC and ACD patterns 4/6=66.7%).

DISCUSSION

This study demonstrated the ability for five abundant coral species (*A. cytherea*, *A. pulchra*, *P. damicornis*, *P. cactus* and *P. rus*) from Moorea to associate with any of the *Symbiodinium* clades A, C and D (over the A to F tested), except for *P. cactus* never observed in association with clade A. These results are congruent with previous data describing these 3 clades as the major clades inhabiting in scleractinian corals (Van Oppen et al., 2005). In contrast, while the clade B was commonly reported in Caribbean corals (Rowan et al., 1997; Diekmann et al., 2003; Pettay & Lajeunesse, 2007; Cunning, Silverstein & Baker, 2015) it was rarely reported in corals from Central Pacific.

This study recorded, for the first time in French Polynesia, the association of *Symbiodinium* clade B with corals (Magalon, Flot & Baudry, 2007; Putnam et al., 2012). It was detected exclusively as a cryptic population in *P. damicornis* and closely convergent with type B1. Interestingly, among the rare inventoried *Symbiodinium* clade B in corals (e.g. LaJeunesse, 2001; Silverstein, Correa & Baker, 2012; Lee et al., 2016), such an association between sub-clade B1 and *P. damicornis* was already reported in Hawaii (LaJeunesse, 2001). Moreover it overlaps with a recent report in Moorea as a symbiotic organism in the nudibranch *Aeolidiella alba (Wecker, Fournier & Bosserelle, 2015)*. Finally, this finding is highly consistent with the recent study of Lee et al. (2016), in which the sensitive method of qPCR has enabled the discovery of a rare *Symbiodinium* clade B (type B2) stable-established in host tissues of *Alveopora japonica*. Therefore, despite the rarity of reports of coral symbioses specialized with *Symbiodinium* clade

B, our findings argue in favour of the symbiotic status of this particular clade, however, with the assumption of low beneficial physiological properties for host survival as they were exclusively detected at only trace level (e.g. sensibility to thermal stress; Loram et al., 2007).

The qPCR assays revealed that each of the four clades A-D could be detected at least once at a cryptic level (i.e. low-abundance), leading to unreported partnerships between coral species and *Symbiodinium* clades (e.g. *P. rus* with clades A or D). Additionally, it is likely that the missing partnerships between *P. cactus* and clade A or between clade B and *Acropora* species, *P. rus* or *P. cactus* could be due to limits in the sampling effort (*i.e.* 6 corals sampled), or by a selective non-association from the host species (Silverstein, Correa & Baker, 2012; Davy, Allemand & Weis, 2012). The possibility for corals to harbour multi-specific *Symbiodinium* communities that involve cryptic clade(s) should represent a relevant ecological concern for holobiont resistance (Berkelmans & Van Oppen, 2006; Mieog et al., 2007). Indeed, such results support the potential for dynamic strategies (switching *vs*. shuffling) described in the ABH, that could lead to a rapid selective mechanism of tolerant coral-*Symbiodinium* partnerships in response to environmental change (Buddemseier & Fautin, 1993; Baker, 2003).

Despite the observed variability for the five coral species studied that increases the degree of flexibility, each of them harboured faithful partnerships with a specific dominant *Symbiodinium* clade(s). Clade A was exclusively observed (>95%; Fig. 1) in the *Symbiodinium* assemblages associated to both *Acropora* species: *A. cytherea* and *A. pulchra*. Similarly, the other coral species exhibited high selectivity to particular clade(s): either exclusively with C for *P. rus* and with D for *P. damicornis*, or dominated with C for *P. cactus*. These findings were entirely consistent to those reported in the study of Putnam et al. (2012), and therefore, implied a wide range of various fringing reefs (Putnam et al., 2012; Rouzé et al., 2015) at different season (*i.e.* dry season in this study *vs*. April: wet season). The few exceptions of these non-respected stable privileged partnerships for *P. damicornis* or *P. cactus*, could be explained by a punctual dynamic

328 lost of *Symbiodinium* clade (Muscatine, 1973; Yamashita et al., 2011), but unlikely due to a
329 spatial variation in the host colony (e.g. Rowan & Knowlton, 1995) given our repetitive sampling
330 procedure. These common trends return the current classification of coral species as 'specialists'
331 complex (low flexibility: specific to particular symbiont(s)) or 'generalists' (high flexibility:
332 associated to various symbionts). To go further in symbiont richness in corals, similar fine
333 molecular approaches (qPCR, next generation sequencing; see Barbrook, Voolstra & Howe,
334 2014) should be now performed on a wide range of coral species through large geographical
335 partitioning.

336 The stability of coral species with specific dominant clade(s) is congruent with similar
337 observations of previous temporal monitoring (e.g. Thornhill et al., 2006, 2009; Suwa, Hirose &
338 Hidaka, 2008; Rouzé et al., 2016). It is likely that the stable partnerships between coral species
339 and faithful clade(s) are derived from various biological traits (Yost et al., 2013) of coral species
340 as well as different physiological and ecological attributes between *Symbiodinium* clades (Kinzie
341 et al., 2001; Berkelmans & Van Oppen, 2006; Hennige et al., 2009; Baker et al., 2013), and/or to
342 various life history between reef habitats (Penin, Vidal-Dupiol & Adjeroud, 2012; Howells et al.,
343 2013a,b). The high resistance of the genus *Porites* to a variety of stressors should be explained in
344 part by its stable association with *Symbiodinium* type C15 (GenBank reference: xx; Putnam et al.
345 2012): attributed with an evolutionary radiation throughout the Indo-Pacific and to a wide host
346 range (LaJeunesse, 2005; Pochon et al., 2007) and characterized as thermally tolerant
347 (LaJeunesse et al., 2003; Fitt et al., 2009), even more resilient to extreme environmental
348 conditions compared to other clade C types (LaJeunesse et al., 2003). In this way, the preferential
349 association of *P. cactus* with *Symbiodinium* clade C, but likely type C1 (GenBank reference: xx;
350 Putnam et al. 2012), should explain its lower resistance to environmental conditions. In the same
351 way, sensitive branching corals from the genera *Acropora* and *Pocillopora* could be explained in
352 part by their privileged associations with clades A, likely type A1 (Putnam et al. 2012) and A13

(GenBank reference: xx), and D, likely type D1 (GenBank reference: xx; Putnam et al. 2012), respectively, both previously reported in normal conditions as low contributors to host metabolism: growth and reproduction (e.g. Little, Van Oppen & Willis, 2004; Jones & Berkelmans, 2010), or nutrition (Cantin et al., 2009; Baker et al., 2013).

The clade faithfulness of coral species likely represents one of the drivers of a stable mutualism, initially resulting from a selective pressure, that should enhance the benefits of a specific symbiosis by co-evolution (Douglas, 2008; Prada et al., 2014). For example, a recent study reported a biochemical complementarity between genomes of *Acropora digitifera* and *Symbiodinium kawagutii* representing clade F strongly indicative of host-symbiont coevolution (Lin et al., 2015). Interestingly, the specific coral species-*Symbiodinium* clade(s) observed for the five coral species appeared independently to the symbiont transmission mode (Table 1). Yet vertical transmission is supposed to increase the strength and efficiency of a specific symbiosis (Herre et al., 1999; Lesser, Stat & Gates, 2013), by the development of new cellular processes such as the retention of symbionts during transmission (Davy, Allemand & Weis, 2012). Such an evolutionary process should have conduct to the stable association of *P. damicornis* and *P. rus* with mono-clade *Symbiodinium* communities involving type D1 and C15, respectively. Compared to the three other coral species, *P. cactus* and *Acropora spp*., horizontal transmitters, these two coral species displayed less variable clade patterns dominated with 'faithful clades' (Fig. 2). However, it contrasts with the detection of additional clades, at trace levels, in both vertical and horizontal transmitting coral species. This should comfort the recent assumption that low-abundance "background" *Symbiodinium* populations are not necessarily mutualistic but can reflect a punctual relative abundance in the surrounding environment (Lee et al., 2016) such as non-directionally ingested by polyps leading to ephemeral symbiont shifts (LaJeunesse et al., 2009; Stat et al., 2009). Nevertheless, the exclusive absence of clade F in host tissues of the five coral species, despite F being detected in the surrounding environment (data not shown) and

already described as dominant symbiotic organisms of a coral host (*i.e. Alveopora japonica*; Lee et al., 2016), suggest the plausibility of combined controlled process(es). In this way, two opposite selection pressures can be co-occuring: i) optimization of the symbiosis with faithful clade(s) and/or ii) maintenance of the ability to integrate different clades, as a biological process.

Altogether, the findings of this study emphasize the need to further understand whether those *Symbiodinium* spp. present in low abundance have an ecological role for the holobiont through time and to consider the process that permits to find them additionally to the stable symbiosis with dominant faithful clade.

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399 **REFERENCES**

400 Baird AH., Guest JR., Willis BL. 2009. Systematic and Biogeographical Patterns in the
401 Reproductive Biology of Scleractinian Corals. *Annual Review of Ecology, Evolution, and*
402 *Systematics* 40:551–571.

403 Baker AC. 2003. Flexibility and specifictity in coral-algal symbiosis: diversity, ecology, and
404 Biogeography of Symbiodinium. *Annual Review of Ecology, Evolution, and Systematics*
405 34:661–689.

406 Baker DM., Andras JP., Jordán-Garza AG., Fogel ML. 2013. Nitrate competition in a coral
407 symbiosis varies with temperature among Symbiodinium clades. *The ISME journal*
408 7:1248–51.

409 Banaszak T. 2000. The synthesis of mycosporine-like amino acids (MAAs) by cultured,
410 symbiotic dinoflagellates. *Journal of experimental marine biology and ecology* 249:219–
411 233.

412 Barbrook AC., Voolstra CR., Howe CJ. 2014. The Chloroplast Genome of a Symbioinium sp.
413 clade C3 isolate. *Protist* 165:1–13.

414 Berkelmans R., Van Oppen MJH. 2006. The role of zooxanthellae in the thermal tolerance of
415 corals: a “nugget of hope” for coral reefs in an era of climate change. *Proceedings of the*
416 *Royal Society B: Biological Sciences* 273:2305–2312.

417 Buddemeier RW., Fautin DG. 1993. Coral bleaching as an adaptive mechanism. *Bioscience*
418 43:320–326.

419 Cantin NE., Oppen MJH., Willis BL., Mieog JC., Negri a. P. 2009. Juvenile corals can acquire
420 more carbon from high-performance algal symbionts. *Coral Reefs* 28:405–414.

421 Cooper TF., Berkelmans R., Ulstrup KE., Weeks S., Radford B., Jones AM., Doyle J., Canto M.,
422 O’Leary R a., van Oppen MJH. 2011. Environmental factors controlling the distribution
423 of symbiodinium harboured by the coral Acropora millepora on the Great Barrier Reef.
424 *PloS one* 6:e25536.

425 Cunning R., Silverstein RN., Baker AC. 2015. Investigating the causes and consequences of
426 symbiont shuffling in a multi-partner reef coral symbiosis under environmental
427 change. *Proceedings of the Royal Society B: Biological Sciences* 282:4352–4361.

428 Davy SK., Allemand D., Weis VM. 2012a. Cell Biology of Cnidarian-Dinoflagellate Symbiosis.
429 *Microbiology and Molecular Biology Reviews* 76:229-261.

430 Diekmann O, Olsen J., Stam W., Bak R.. 2003. Genetic variation within Symbiodinium clade B
431 from the coral genus Madracis in the Caribbean (Netherlands Antilles). *Coral Reefs*
432 22:29–33.

433 Douglas AE. 2008. Conflict, cheats and the persistence of symbioses. *New Phytologist*
434 177:849–858.

435 Fabina NS., Putnam HM., Franklin EC., Stat M., Gates RD. 2012. Transmission Mode Predicts
436 Specificity and Interaction Patterns in Coral-Symbiodinium Networks. *PLoS One* 7:9.

437 Fitt WK., Gates RD., Hoegh-Guldberg O., Bythell JC., Jatkar a., Grottoli a. G., Gomez M., Fisher
438 P., Lajuenesse TC., Pantos O., Iglesias-Prieto R., Franklin DJ., Rodrigues LJ., Torregiani
439 JM., van Woesik R., Lesser MP. 2009. Response of two species of Indo-Pacific corals,

Porites cylindrica and Stylophora pistillata, to short-term thermal stress: The host does matter in determining the tolerance of corals to bleaching. *Journal of Experimental Marine Biology and Ecology* 373:102–110.

Hennige SJ., Suggett DJ., Warner ME., McDougall KE., Smith DJ. 2009. Photobiology of Symbiodinium revisited : bio-physical and bio-optical signatures. *Coral Reefs* 28:179–195.

Herre EA., Knowlton N., Mueller UG., Rehner SA. 1999. The evolution of mutualisms: Exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution* 14:49–53.

Howells EJ., Willis BL., Bay LK., van Oppen MJH. 2013a. Spatial and temporal genetic structure of Symbiodinium populations within a common reef-building coral on the Great Barrier Reef. *Molecular Ecology* 22:3693–3708.

Howells EJ., Berkelmans R., van Oppen MJH., Willis BL., Bay LK. 2013b. Historical thermal regimes define limits to coral acclimatization. *Ecology* 94:1078–88.

Jones A., Berkelmans R. 2010. Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types. *PloS one* 5:e10437.

Kayal M., Vercelloni J., Lison de Loma T., Bosserelle P., Chancerelle Y., Geoffroy S., Stievenart C., Michonneau F., Penin L., Planes S., Adjeroud M. 2012. Predator crown-of-thorns starfish (Acanthaster planci) outbreak, mass mortality of corals, and cascading effects on reef fish and benthic communities. *PloS one* 7:e47363.

Kinzie RA., Takayama M., Santos SR., Coffroth MA. 2001. The Adaptive Bleaching Hypothesis : Experimental Tests of Critical Assumptions. *Biological Bulletin* 200:51–58.

LaJeunesse TC. 2001. Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus Symbiodinium using the ITS region: in search of a“ species” level marker. *Journal of Phycology* 37:866–880.

LaJeunesse TC., Loh WKW., Van Woesik R., Hoegh-Guldberg O., Schmidt GW., Fitt WK. 2003. Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. *Limnology and Oceanography* 48:2046–2054.

LaJeunesse TC. 2005. “Species” radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene- Pliocene transition. *Molecular biology and evolution* 22:570–581.

LaJeunesse TC., Smith RT., Finney J., Oxenford H. 2009. Outbreak and persistence of opportunistic symbiotic dinoflagellates during the 2005 Caribbean mass coral “bleaching” event. *Proceedings of the Royal Society B-Biological Sciences* 276:4139–4148.

LaJeunesse TC., Smith R., Walther M., Pinzon J., Pettay DT., McGinley M., Aschaffenburg M., Medina-Rosas P., Cupul-MagaÒa AL., PÈrez AL. 2010. Host-symbiont recombination versus natural selection in the response of coral-dinoflagellate symbioses to environmental disturbance. *Proceedings of the Royal Society B* 277:2925–2934.

LaJeunesse TC., Wham DC., Pettay DT., Parkinson JE., Keshavmurthy S., Chen CA. 2014. Ecologically differentiated stress-tolerant endosymbionts in the dinoflagellate genus Symbiodinium (Dinophyceae) Clade D are different species. *Phycologia* 53:305–319.

Lee MJ., Jeong HJ., Jang SH., Lee SY., Kang NS., Lee KH., Kim HS., Wham DC., LaJeunesse TC.

2016. Most Low-Abundance "Background" Symbiodinium spp. Are Transitory and Have Minimal Functional Significance for Symbiotic Corals. *Microbial Ecology*.

Lesser MP., Stat M., Gates RD. 2013. The endosymbiotic dinoflagellates (Symbiodinium sp.) of corals are parasites and mutualists. *Coral Reefs* 32:603–611.

Lin S., Shifeng C., Song B., Zhong X., Lin X., Li W., Li L., Zhang Y. 2015. The Symbiodinium kawagutii genome illuminates dinoflagellate gene expression and coral symbiosis. *Science* 240:13201–13202.

Little AF., Van Oppen MJH., Willis BL. 2004. Flexibility in algal endosymbioses shapes growth in reef corals. *Science* 304:1492–1494.

Loram JE., Boonham N., O'Toole P., Trapido-Rosenthal HG., Douglas AE. 2007. Molecular quantification of symbiotic dinoflagellate algae of the genus Symbiodinium. *The Biological Bulletin* 212:259.

Magalon H., Flot J-F., Baudry E. 2007. Molecular identification of symbiotic dinoflagellates in Pacific corals in the genus Pocillopora. *Coral Reefs* 26:551–558.

McClanahan TIRMCC., Teweberhan MEA., Uhando CHAM., Aina JOM. 2007. Effects of climate and seawater temperature variation on coral bleaching and mortality. *Ecological Monographs* 77:503–525.

Mieog JC., van Oppen MJH., Cantin NE., Stam WT., Olsen JL. 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.

Mieog JC., Van Oppen MJH., Berkelmans R., Stam WT., Olsen JL. 2009. Quantification of algal endosymbionts(Symbiodinium) in coral tissue using real-time PCR. *Molecular Ecology Resources* 9:74–82.

Muscatine L. 1973. Nutrition of corals Biology and Geology of Coral Reefs. :77–115.

Muscatine L., Porter J. 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *Bioscience* 27:454–460.

Pandolfi JM., Connolly SR., Marshall DJ., Cohen AL. 2011. Projecting coral reef futures under global warming and ocean acidification. *Science (New York, N.Y.)* 333:418–22.

Penin L., Adjeroud M., Schrimm M., Lenihan HS. 2007. High spatial variability in coral bleaching around Moorea (French Polynesia): patterns across locations and water depths. *Comptes rendus biologies* 330:171–81.

Penin L., Vidal-Dupiol J., Adjeroud M. 2012. Response of coral assemblages to thermal stress: are bleaching intensity and spatial patterns consistent between events? *Environmental Monitoring and Assessment* 185:5031–5042.

Pettay DT., Lajeunesse TC. 2007. Microsatellites from clade B Symbiodinium spp. specialized for Caribbean corals in the genus Madracis. *Molecular Ecology Notes* 7:1271–1274.

Pochon X., Garcia-Cuetos L., Baker a. C., Castella E., Pawlowski J. 2007. One-year survey of a single Micronesian reef reveals extraordinarily rich diversity of Symbiodinium types in soritid foraminifera. *Coral Reefs* 26:867–882.

Pochon X., Putnam HM., Gates RD. 2014. Multi-gene analysis of Symbiodinium dinoflagellates: a perspective on rarity, symbiosis, and evolution. *PeerJ* 2:e394.

Prada C., McIlroy SE., Beltrán DM., Valint DJ., Ford S a., Hellberg ME., Coffroth MA. 2014.

525 Cryptic diversity hides host and habitat specialization in a gorgonian-algal symbiosis.
526 *Molecular Ecology* 23:3330–3340.

527 Putnam HM., Stat M., Pochon X., Gates RD. 2012. Endosymbiotic flexibility associates with
528 environmental sensitivity in scleractinian corals. *Proceedings of the Royal Society B:*
529 *Biological Sciences* 279:4352–4361.

530 Richter C., Roa-Quiaoit H., Jantzen C., Al-Zibdah M., Kochzius M. 2008. Collapse of a new
531 living species of giant clam in the Red Sea. *Current biology : CB* 18:1349–54.

532 Rouzé H., Lecellier G., Langlade M., Planes S., Berteaux-Lecellier V. 2015. Fringing reefs
533 exposed to different levels of eutrophication and sedimentation can support the same
534 benthic communities. *Marine pollution bulletin* 92:212–221.

535 Rouzé H., Lecellier G., Saulnier D., Berteaux-Lecellier V. 2016. Symbiodinium clades A and D
536 differentially predispose Acropora cytherea to disease and Vibrio spp. colonization.
537 *Ecology and Evolution* 6:560–572.

538 Rowan R., Knowlton N., Baker A., Jara J. 1997. Landscape ecology of algal symbionts creates
539 variation in episodes of coral bleaching. *Nature* 388:265–269.

540 Rowan R., Knowlton N. 1995. Intraspecific diversity and ecological zonation in coral-algal
541 symbiosis. *Proceedings of the National Academy of Sciences of the United States of*
542 *America* 92:2850–2853.

543 Sampayo EM., Ridgway T., Bongaerts P., Hoegh-Guldberg O. 2008. Bleaching susceptibility
544 and mortality of corals are determined by fine-scale differences in symbiont type. *PNAS*
545 105:10444–10449.

546 Schmidt-Roach S., Miller KJ., Lundgren P., Andreakis N. 2014. With eyes wide open: a
547 revision of species within and closely related to the Pocillopora damicornis species
548 complex (Scleractinia; Pocilloporidae) using morphology and genetics. *Zoological*
549 *Journal of the Linnean Society* 170:1–33.

550 Silverstein RN., Correa AMS., Baker AC. 2012. Specificity is rarely absolute in coral-algal
551 symbiosis: implications for coral response to climate change. *Proceedings of the Royal*
552 *Society B: Biological Sciences*:2609–2618.

553 Stat M., Loh WKW., LaJeunesse TC., Hoegh-Guldberg O., Carter DA. 2009. Stability of coral-
554 endosymbiont associations during and after a thermal stress event in the southern
555 Great Barrier Reef. *Coral Reefs* 28:709–713.

556 Stat M., Carter D., Hoeghguldberg O. 2006. The evolutionary history of Symbiodinium and
557 scleractinian hosts—Symbiosis, diversity, and the effect of climate change. *Perspectives*
558 *in Plant Ecology, Evolution and Systematics* 8:23–43.

559 Suwa R., Hirose M., Hidaka M. 2008. Seasonal fluctuation in zooxanthellar genotype
560 composition and photophysiology in the corals Pavona divaricata and P. decussata.
561 *Marine Ecology Progress Series* 361:129–137.

562 Thornhill DJ., LaJeunesse TC., Kemp DW., Fitt WK., Schmidt GW. 2006. Multi-year, seasonal
563 genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching
564 reversion. *Marine biology* 148:711–722.

565 Thornhill DJ., Xiang Y., Fitt WK., Santos SR. 2009. Reef Endemism, Host Specificity and
566 Temporal Stability in Populations of Symbiotic Dinoflagellates from Two Ecologically
567 Dominant Caribbean Corals. *PLoS One* 4:12.

568 Ulstrup KE., Van Oppen MJH. 2003. Geographic and habitat partitioning of genetically
569 distinct zooxanthellae (Symbiodinium) in Acropora corals on the Great Barrier Reef.
570 *Molecular Ecology* 12:3477–3484.

571 Wecker P., Fournier A., Bosserelle P. 2015. Dinoflagellate diversity among nudibranchs and
572 sponges from French Polynesia : Insights into associations and transfer. *Comptes*
573 *Rendus Biologies* 338:278–283.

574 Wilcox TP. 1998. Large-Subunit Ribosomal RNA Systematics of Symbiotic Dinoflagellates:
575 Morphology Does Not Recapitulate Phylogeny. *Molecular Phylogenetics and Evolution*
576 10:436–448.

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

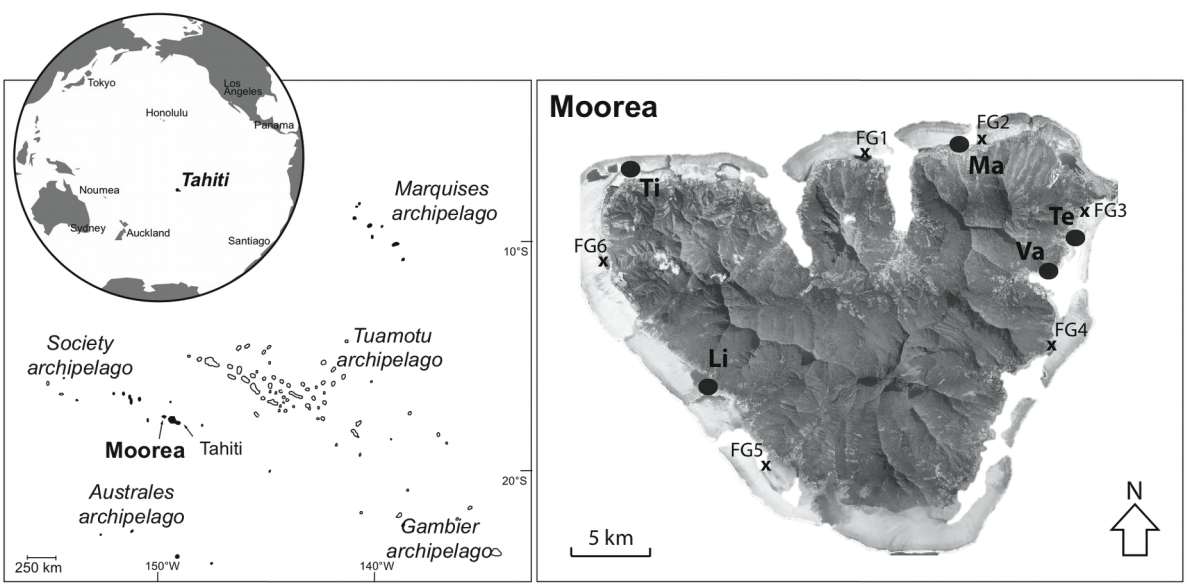
581 van Oppen MJH., Mieog JC., Sánchez CA., Fabricius KE. 2005. Diversity of algal
582 endosymbionts (zooxanthellae) in octocorals: The roles of geography and host
583 relationships. *Molecular Ecology* 14:2403–2417.

584 van Woesik R., Sakai K., Ganase a., Loya Y. 2011. Revisiting the winners and the losers a
585 decade after coral bleaching. *Marine Ecology Progress Series* 434:67–76.

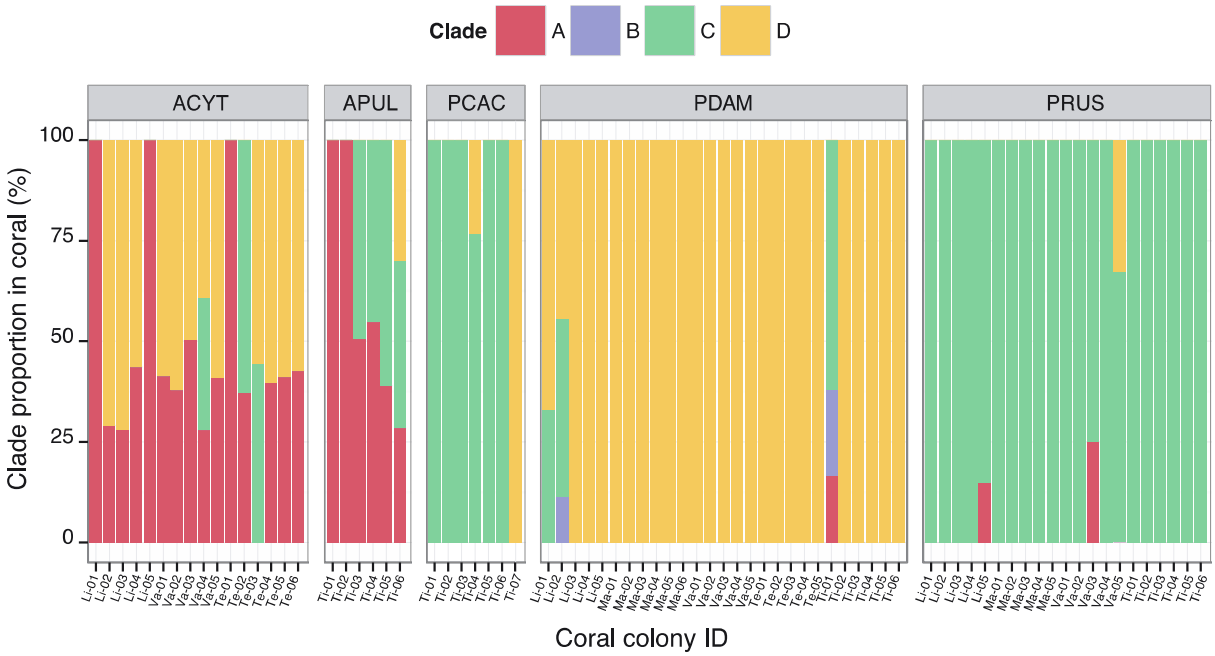
586 Yamashita H., Suzuki G., Hayashibara T., Koike K. 2011. Do corals select zooxanthellae by
587 alternative discharge? *Marine biology* 158:87–100.

588 Yost DM., Wang L., Fan T., Chen C., Lee RW., Sogin E., Gates RD. 2013. Diversity in skeletal
589 architecture influences biological heterogeneity and Symbiodinium habitat in corals.
590 *Zoology* 116:262–269.

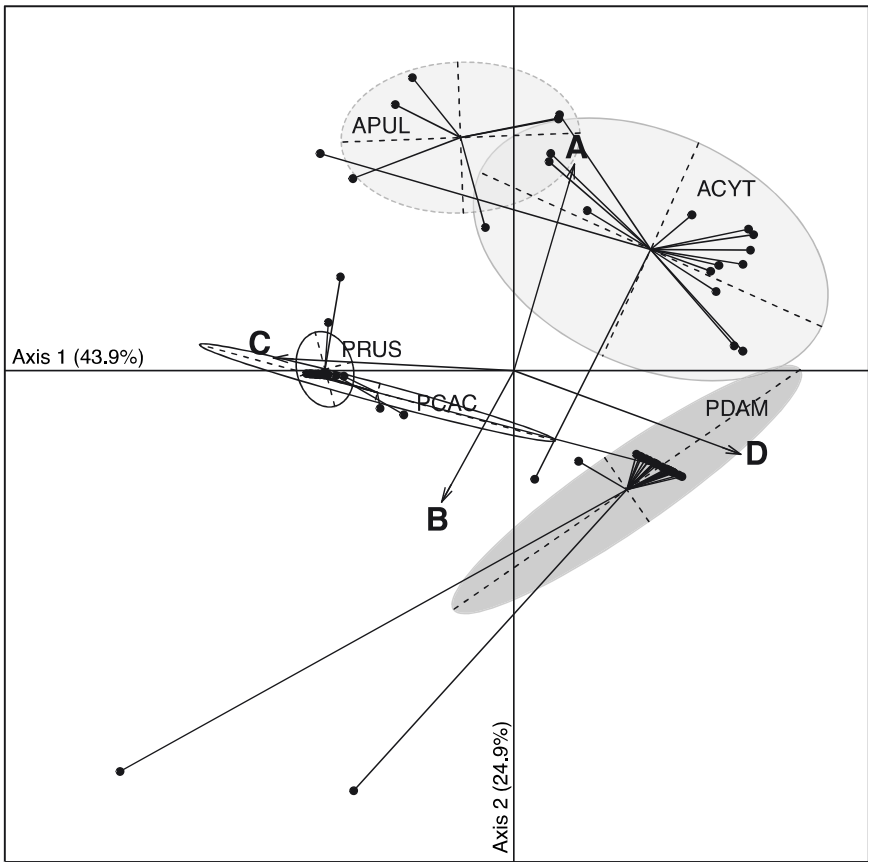
591 **Figure1** Localization of Moorea island (Archipelago of society, French Polynesia) and the
592 locations of the fringing reefs studied in this study (black circle). Locations of previous fringing
593 reefs where *Symbiodinium* have been investigated in the study of Putnam et al. (2012) are
594 indicated by cross symbols. Vaiare (Va), Teavaro (Te), Maharepa (Ma), Tiahura (Ti) and Linereva
595 (Li).



596 **Figure2** Quantitative composition of different symbiotic patterns observed in association with
597 ACYT: *A. cytherea*, APUL: *A. pulchra*, PCAC: *P. cactus*, PDAM: *P. damicornis* and PRUS:
598 *P. rus*.



599 **Figure3** Spatio-temporal multivariate analysis of clade A-D quantifications converted in 28S
600 copy number. Axis 1 and 2 of the discriminant analysis of principal component (DAPC)
601 according to the five coral species: *A. cytherea*, *A. pulchra*, *P. cactus*, *P. damicornis* and *P. rus*.



602 **Table 1** Biological traits of different coral species: *A. cytherea*, *A. pulchra*, *P. cactus*,
603 *P. damicornis* and *P. rus*.

Genus	Specie	N	Transmission	Morphology	Sensitivity
Acropora	*A. pulchra*	6	Horizontal	Branching (steel)	High
	A. cytherea	16	Horizontal	tabular	
Pocillopora	*P. damicornis*	27	Vertical	branching	Moderate
Pavona	*P. cactus*	7	Horizontal	folliaced	Moderate
Porites	*P. rus*	21	Vertical	Massive-branching	Low

604 **Table 2** Comparative census of *Symbiodinium* clades and types associated to common coral
605 species from Moorea *A. cytherea*, *A. pulchra*, *P. damicornis*, *P. cactus* and *P. rus* detected in
606 previous report of Putnam et al. 2012 [1] *vs*. the present study.

Coral species	**Previous report** [1]		**Present study**		
	clade(s)	type	clades	* type	* accession nos
A. cytherea	A, D	A1, D1	A, C*, D	* C1	xx
A. pulchra	A, D	A1, D1	A, C*, D		
P. damicornis	A, C, D	DA, A1, C15	A, B*, C, D	* B1	B : xx
P. rus	C	C15	A*, C**, D*	*: A13, D1; C15, **:Cx	A :xx, C: xx; D: xx
P. cactus	C	C1, C3, C45	D*	*: D1, **Cx	C : xx, D: xx

607 ** novel detected clade from this study*
608 *** new type of previously reported clade*

609 **Sequences for submission in GenBank**

610 > *Sym B_ isolated from P. damicornis*
611 TCACATGTCGTGCTGAGATTGCTGTGGGTCTTTGTGAGCCTTGAGCATGTAAGCGCAAGCTGACTGCTTA

612 TG-TGTGAGCATTTACCC-GCAGTGTTTCTCAGCATGCGAG