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Metacommunity ecology of Symbiodiniaceae hosted by the coral *Galaxea fascicularis*

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ABSTRACT: Coral–algae symbiosis represents the trophic and structural basis of coral reef ecosystems. However, despite global threats to coral reefs and the dependence of coral health and stress resistance upon such mutualisms, little is known about the community ecology of endosymbiotic Symbiodiniaceae. Concepts and methods from metacommunity ecology may be used to help us understand the assembly and stability of symbiont communities and the mutualisms they comprise. In this study, we sampled colonies of the symbiont-generalist coral *Galaxea fascicularis* in southwestern Japan and assessed the effects of environmental and host factors on Symbiodiniaceae community composition, while simultaneously exploring residual correlations among symbiont types that may reflect non-random assembly processes such as species interactions. We metabarcoded the Symbiodiniaceae ribosomal internal transcribed spacer 2 (ITS2) region and characterized the endosymbiotic community using 2 different OTU identity cut-offs, and analyzed them with generalized dissimilarity modeling and joint species distribution modeling. We found that Symbiodiniaceae form discrete communities characterized by the dominance of ITS2 types C1, C21a, or D1, that are each associated with a different suite of co-occurring background types and tend to exclude each other in an endosymbiotic community. The communities showed modest responses to temperature, water depth, host genotype, polyp size, and bleaching status, and there was local sequence variation within the ITS2 types. After accounting for the effects of those variables, residual correlations remained in community composition, pointing to the possibility that Symbiodiniaceae community assembly in corals may be structured by interspecific competitive or facilitating interactions rather than only exogenous variables.

KEY WORDS: Symbiodiniaceae · Metacommunity · Joint species distribution model · ITS2 · Metabarcoding · Minimum entropy decomposition · *Galaxea fascicularis*

1. INTRODUCTION

Mutualisms between multicellular organisms and microbes form the basis of some of the most diverse ecosystems on the planet (Boucher et al. 1982). In such systems, microbial symbionts form assemblages of potentially interacting individuals and species—ecological communities—within each host. The hosts

are often distributed across space and environmental gradients, providing metacommunity structure to the local microbial assemblages (Leibold et al. 2004). Thus, the application of questions, concepts, and methodologies from metacommunity ecology to the study of symbiont assemblages may help illuminate key aspects of their ecology, organization, and stability in the face of perturbation (Mihaljevic 2012).

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Perhaps the most fundamental question of community ecology is how individuals and species assemble into communities. In most terrestrial and marine systems, environmental gradients given by climatic variation underlie variation in community composition to some degree, because climate strongly affects the physiology and fitness of most organisms (Simberloff & Dayan 1991). However, positive and negative biotic interactions also influence community structure (Chesson 2000). After accounting for the effects of the environment on individual species, there may be residual correlations between pairs of species that identify modules of interacting species—different communities or subcommunities that repeatedly assemble in space. Here, we analyzed the metacommunity composition of Symbiodiniaceae in corals using metabarcoding and a joint species distribution modeling framework.

The Scleractinia–Symbiodiniaceae symbiosis may be viewed as the structural and trophic fundament of coral reef ecosystems that are renowned for their incredible diversity (Roberts et al. 2002). Collapse of this symbiosis due to environmental stressors and diseases—coral bleaching—has repeatedly caused entire ecosystems to break down (Hughes et al. 2017). The resistance of corals to bleaching is known to be affected by the composition of endosymbiotic Symbiodiniaceae communities (Sampayo et al. 2008). We evaluated the extent to which composition and turnover of the Symbiodiniaceae community hosted in individual corals are driven by environmental and host factors, and after accounting for those, whether there are residual correlations indicating clusters of positively and negatively co-occurring variants. Such correlations could indicate that there are alternative stable symbiont communities that can form within coral polyps that may respond differently to environmental stressors responsible for mutualism breakdown and coral bleaching.

Symbiodiniaceae are a family of dinoflagellates classified into 9 ecologically distinct and phylogenetically highly divergent genera (previously 'clades' denominated A–I), based upon the 18S rDNA region (Rowan & Powers 1991, LaJeunesse 2001, Pochon et al. 2004, Pochon & Gates 2010). This family was previously known as the genus *Symbiodinium* and has recently been lifted to family level for their high level of molecular divergence (LaJeunesse et al. 2018). Scleractinia most often host the genera *Breviolum* J.E. Parkinson & LaJeunesse, *Cladocopium* LaJeunesse & H.J. Jeong, and *Durusdinium* LaJeunesse (formerly clades B, C, D; Baker 2003), with a predominance of *Cladocopium* in the Indo-Pacific to which they main-

tain intimate mutualistic relationships (Muscatine & Porter 1977). The genera (previously 'clades') were further classified into hundreds of 'types' based on sequence variation of the ribosomal internal transcribed spacer 2 (ITS2), which were due to host specializations and varying geographic and depth distributions considered to represent evolutionarily significant units that approximate species (e.g. LaJeunesse et al. 2003, LaJeunesse 2005); the concordance with multiple other genetic markers has also led to formal species descriptions (LaJeunesse et al. 2012, 2014, Parkinson & Coffroth 2015). The community in a host individual usually consists of one dominant type and several minor background types (Mieog et al. 2007). Background types may influence recovery after a bleaching event (Berkelmans & van Oppen 2006) and there is increasing evidence that they play a significant role in the stability of a regularly structured community (Boulotte et al. 2016, but see Lee et al. 2016, Ziegler et al. 2018).

Generally, the physical environment is expected to have a strong influence on *in hospite* Symbiodiniaceae community assembly. For example, the dominant Symbiodiniaceae types within host species vary with latitude, seasons, and depths (reviewed in Baker 2003, Bongaerts et al. 2015). The host as part of the endosymbiotic environment also has a significant influence on community composition; this is apparent from host–symbiont specifications, including between morphologically cryptic host lineages (Pinzon et al. 2013, Warner et al. 2015), as well as from the host health status or degree of bleaching, which directly mirror the *in hospite* Symbiodiniaceae community (Rowan 2004, Chen et al. 2005). Coral skeletal morphology was also shown to affect the Symbiodiniaceae community since photosynthetic activity varies between Symbiodiniaceae genera and types and is linked to calcification and growth rate (Little et al. 2004, Yost et al. 2013, Diaz-Almeyda et al. 2017). These observations have been made primarily on the dominant Symbiodiniaceae types in a community, and variation according to the environment or host impacts on the level of Symbiodiniaceae ITS2 types are only beginning to emerge (Parkinson et al. 2016, Diaz-Almeyda et al. 2017). Community-level Symbiodiniaceae data have only recently become available at sufficient resolution (e.g. Cunning et al. 2015, Gorospe et al. 2015, Boulotte et al. 2016, Ziegler et al. 2018) due to the decreasing cost of next-generation sequencing technology, and much remains unknown regarding their assembly processes, such as their individual roles in and on a symbiotic community.

While advances in next-generation sequencing have enabled characterizations of symbiont commu-

nities, new statistical methods facilitate both the evaluation of environmental effects on species distributions and residual correlations between species. In particular, joint species distribution modeling is an emerging paradigm that extends single species distribution models to include the potential biotic affects among species (Warton et al. 2015a, 2016, Ovaskainen et al. 2017). Unlike other common approaches for community analysis that are based on co-occurrence patterns alone, such as network analysis, joint species distribution models are able to correct for co-occurrences caused by similar environmental preferences. These residual or ‘latent’ correlations reflect patterns of species that do or do not tend to occur together after accounting for environmental filtering processes, and can potentially reflect positive and negative biotic interactions among species (Warton et al. 2015b). This approach of jointly modeling occurrence of multiple species complements other approaches that seek to explain community-level beta-diversity as a function of environmental or other factors, such as generalized dissimilarity modeling (GDM).

In this study, we used deep sequencing technology, joint species distribution modeling, and GDM to analyze the metacommunity ecology of endosymbiotic Symbiodiniaceae ITS2 types on the coral *Galaxea fascicularis* (Linnaeus, 1767), across the Nansei Islands in southwestern Japan. ITS2 was chosen as a genetic marker as it is the most commonly used descriptor of Symbiodiniaceae diversity. Despite problems related to its multicopy nature (Arif et al. 2014), it remains an essential marker to contextualize new studies with the large body of existing data (Cunningham et al. 2017). More recent research also showed that ITS2 may sometimes over-aggregate lineages (LaJeunesse & Thornhill 2011, Reimer et al. 2017), which is why we applied multiple clustering thresholds. By finely resolving the ITS2 variation between and within individuals using deep-sequencing and minimum entropy decomposition (Eren et al. 2015), differentiation within what would cluster as a single ITS2 type using traditional operational taxonomic unit (OTU) approaches may be revealed.

Galaxea fascicularis is a suitable model system to investigate Symbiodiniaceae metacommunity ecology, since it is a widely distributed generalist species acquiring symbionts from the settlement location (Baird et al. 2009) and occurs at various depths on the reef. There are several genetic lineages within this species with little morphological differentiation (Nakajima et al. 2016). Associations with *Cladocopium* ITS2 types C1, C1b, C2r, C21, C21a, C27, C3, C3d, C3u, C40, and C161, and *Durusdinium* types

D1, D2, and D17 have been reported (GeoSymbio: Franklin et al. 2012, Zhou et al. 2012, 2017, Tong et al. 2017), indicating a rather flexible relationship potentially varying according to exogenous characteristics. We specifically evaluated the extent to which geographic location, temperature, depth, host lineage, polyp size, and bleaching status predict the distributions of Symbiodiniaceae types and communities between colonies of *Galaxea fascicularis*. After accounting for these factors, we further evaluated the extent to which residual modules of ITS2 types display non-random positive and negative associations—alternative communities that can form in the coral polyps. This type of unexplained metacommunity structure could be the result of uncharacterized exogenous variables, but also could be an indication that communities are shaped by endogenous dynamics such as facilitating and competitive interactions among species.

2. MATERIALS AND METHODS

2.1. Field sampling

Coral colonies of the taxon *Galaxea fascicularis* L. were collected from 6 locations along a latitudinal gradient in the Nansei Islands (Fig. 1, Table 1; permit nos. 26–62, 27–64). A total of 1–2 polyps from the central part of a coral colony were sampled. They were collected by SCUBA divers from the forereef, except in Okinawa Main Island (hereafter referred to as Oki-

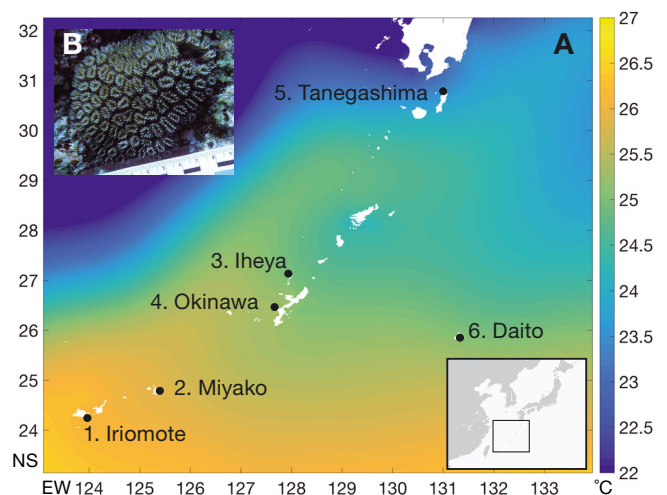


Fig. 1. (A) Mercator projected map of sampling sites along the Nansei Islands. Annual mean sea surface temperature is indicated as background color (data retrieved from WOA13); numbers refer to location IDs in Table 1. (B) Example of *Galaxea fascicularis* sampled in Iheya

Table 1. Sampling information and metadata for specimens of the coral *Galaxea fascicularis* that were collected from 6 different sites along the Nansei Islands, Japan. SST: sea surface temperature; NA: not available. Sites 5 and 6 had limited metadata (no colony photo or *in situ* temperature)

ID	1	2	3	4	5	6
Location	Miyako (Yoshino)	Iheya (north cape)	Tanegashima	Daito (Minami and Kita)	Okinawa (Zanpa)	Iriomote (Haemida)
Latitude (°N)	24.74841	27.0927	30.827102	25.87795	26.43889	24.268333
Longitude (°E)	125.44599	128.01216	131.035352	131.21427	127.71111	123.82972
Collection date	20 Jul 2015	29 Jul 2015	14 Sept 2015	11 Nov 2015	16 Nov 2013	24 Aug 2014
Depth (m)	6.7–19.8	9.3–12.6	6.7–11.4	14.8–23.9	~1–2	~3–5
No. of samples	10	17	10	10	10	10
SST annual mean (°C)	25.9	24.6	22.9	25.6	25.6	26.1
SST collection month mean (°C)	29.4	27	28	25.9	26.3	28.4
Temperature <i>in situ</i> (°C)	28	28	27	26	NA	NA
Host genotype	L, S	L, S, L+	L, S	L, S	L, S	L, S
Colony photo	Yes	Yes	Yes	Yes	No	No

nawa), where samples were picked from the inner reef during walks at low tide. Collections since 2015 from the locations at Miyako, Iheya, Tanegashima, and Daito Islands include photo documentation, precise depth, and *in situ* temperature recording. Earlier collections from Iriomote and Okinawa have no associated metadata but were included to explore geographic variation for Symbiodiniaceae community composition. In total, 67 colonies were selected for Symbiodiniaceae ITS2 metabarcoding. Remaining collection material was deposited at the Marine Biophysics Unit at the Okinawa Institute of Science and Technology (Table S1 in the Supplement at www.int-res.com/articles/suppl/m633p071_supp.pdf).

2.2. DNA sequencing and processing

DNA was extracted using the Qiagen DNeasy Blood and Tissue DNA extraction kit following the manufacturer's protocol. Samples were deep-sequenced in the Symbiodiniaceae ITS2 region using the Illumina MiSeq platform. The primers IlluminaAmplicon-ITS-Dino-forward 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG AAT TGC AGA ACT CCG TG-3' and IlluminaAmplicon-its2rev2-reverse 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GCC TCC GCT TAC TTA TAT GCT T-3' (modified from Pochon et al. 2001) were used for amplification of the Symbiodiniaceae ITS2 region. The 300 × 300 bp paired-end sequencing was conducted by the Okinawa Institute of Science and Technology sequencing center on the Illumina MiSeq sequencing platform with v3 chemistry. The samples were sequenced in 2 sets; the first contained samples from Iriomote and Okinawa, and the second contained all

other samples. The raw reads were quality filtered and trimmed with Trimmomatic software (Bolger et al. 2014) to have a minimal length of 80 bases and a minimal phred33 >15 over 4 subsequent bases, then merged with their pairs using USEARCH (Edgar 2013). A total of 10.9 M reads were gained, and after quality filtering and merging, 4.2 M sequences remained (average 56 k sample⁻¹). The samples from the first library were randomly subsampled to contain a comparable number of paired sequences as the samples from the second library (100 000 paired sequences sample⁻¹). Raw reads were submitted to the NCBI Sequence Read Archive (BioSample IDs SRR7528926 - 93).

2.3. Environment and host characteristics

Factors related to temperature and light intensity were chosen to characterize the environment, since these are well known to affect the distribution of Symbiodiniaceae (Baker 2003). During collection trips in 2015 and later (Table 1), water depth and *in situ* temperature were recorded with a dive computer (Scubapro Chromis; precision 1°C). Depth was recorded for each sample during the dive and *in situ* temperature was retrieved as average temperature of the dive (and site) after the dive. Measured depths were corrected for tidal levels by subtracting the tidal level at the time of sampling from the dive computer reading. Sea surface temperature (SST) was also retrieved from the World Ocean Atlas 13 (Locarnini et al. 2013) at the site level. Annual mean SST was calculated from monthly values based upon the most recent available data (2005–2012; Fig. 1, Table S1).

Genetic lineages of hosts were assessed based on mitochondrial and nuclear data. There are at least 3 morphologically cryptic lineages (L, S or L+) within *Galaxea fascicularis* in the Nansei Islands, which differ in the length of a mitochondrial non-coding region (Watanabe et al. 2005, Nakajima et al. 2015, 2016). Lineage identity was determined by fragment length analysis of this mitochondrial region following Nakajima et al. (2015) and confirmed with nuclear RAD data for all specimens (Wepfer 2018) except for Daito PW246 (S), Daito PW236 (S), Iheya PW121 (L+), Iheya PW116 (L+), Iheya PW120 (L+), and Tanega PW196 (L).

The host phenotype was characterized for samples with available photographs (Table 1). Since the presence of Symbiodiniaceae reflects colony health, the degree of bleaching was recorded as 'healthy' if colonies showed no sign of bleaching, 'partially bleached' if individual polyps (usually located at the margins of the colony) were bleached, and 'bleached' if most of the colony was white, using the white of a colored scale as a reference (Fig. S1). Fluorescent green pigments produced by the host itself and the naturally lighter pigmentation in some colonies were disregarded in this assessment. To test a potential effect on skeleton growth, maximal polyp diameter was measured for 3–5 centrally located polyps and averaged per colony using Fiji software (Schindelin et al. 2012).

2.4. OTU delimitation and identification

The delimitation of biologically meaningful OTUs is a challenge for any microbial study. Most commonly, sequences are grouped using heuristic clustering techniques according to predetermined percent identity radii, and for ITS2 of Symbiodiniaceae, a conservative identity threshold of 97% is often chosen to avoid splitting intragenomic variants (Arif et al. 2014). However, these clustering approaches are sometimes unable to detect low-abundance diversity, and the *a priori* setting of an identity radius can be arbitrary (Callahan et al. 2017). In Symbiodiniaceae, too, the threshold of 97% may lump ecologically distinct types (Sampayo et al. 2007, LaJeunesse & Thornhill 2011), and higher percentage radii of 98–100% have also been applied (Smith et al. 2017, Kenkel & Bay preprint doi:10.1101/067322). We therefore decided to investigate our data with 2 different identity thresholds, first using a heuristic clustering approach applying the maximally conservative 97% clustering threshold in order to avoid

splitting of intragenomic variants (Arif et al. 2014), and also using the minimum entropy decomposition (MED) pipeline (Eren et al. 2015) to describe ITS2 diversity at the level of highly resolved nodes (99.11% identity radius). All OTUs and MED nodes were identified using a database provided by Cunningham et al. (2015) using the same naming. Respective accession numbers of these reference sequences are given in Table S2. Details of the 2 clustering approaches and identification procedures are in the Supplement.

2.5. Community analysis

The raw community matrix of the OTUs and MED nodes (symbionts \times coral samples) were visualized as heatmaps using the 'pheatmap' package (Kolde 2015), after standardization and \log_{10} transforming non-zero counts of the matrix. The MED nodes were then rarefied using the 'rarefy' function of the R 'GUniFrac' package, applying the minimum abundance within a sample as a threshold before computing Bray-Curtis dissimilarity between communities. Bray-Curtis dissimilarity was visualized with a 2-dimensional non-metric multidimensional scaling using the R package 'vegan' (Oksanen et al. 2012, R Core Team 2015).

To evaluate the overall explanatory power of exogenous variables such as climate and host characteristics on community structure, we used GDM. We modeled Symbiodiniaceae community composition between coral samples by fitting a GDM to Bray-Curtis community distances using the 'gdm' package (Ferrier et al. 2007) in R. GDMs are extensions of regressions based on distance matrices, but they allow for non-linear relationships between response and predictor variables (Ferrier et al. 2007). GDM was performed 2 times; once on the full sample set including only the predictor variables mean SST, water depth, location, and host lineage, and once on the subset of 47 samples that additionally included the predictors *in situ* temperature, host polyp size, and bleaching status. Sequencing depth (total count of raw FASTQ sequences per sample) was also included to control for a potential effect of sampling effort. The nominal factor 'host lineage' was transformed to numerical principal components by first expanding to 2 dummy variables and then computing 2 principal components using 'pcaMethods' in R, as suggested by the author of the package (Ferrier et al. 2007). In addition, geographic distance was included in the model to account for the potential influ-

ence of distance per se on the difference in community composition (unlike the factor location, which was included to account for unmeasured potential differences in the environment between locations, such as geology, syntopic fauna, or geologic history). Significance and relative importance of the variables were tested with 100 permutations. Parameter splines of the GDM models are given in Fig. S2.

To assess latent correlations between pairs of species while correcting for measured environmental factors, we used a joint species distribution model based on the latent variable modeling approach implemented in the 'boral' package (Hui 2016). Latent variables may be interpreted as the influence of unmeasured environmental predictors; for example, correlations in latent variables imply shared character states of unmeasured predictors in the environment, or correlations in occurrence resulting from biotic species interactions (Warton et al. 2015a, Björk et al. 2018). Joint species distribution models were fitted to both the OTUs clustered to 97% and the more finely resolved MED nodes for analyzing correlations of species and correlations of potential intragenomic variants, respectively, based on the complete sample set (67 specimens). Only OTU or MED nodes that were present in at least 2 samples were included. As covariates, we used the (standardized) continuous variables SST and water depth and the factor host lineage, which were entered into the model via 2 dummy variables. In contrast to the GDM, geographic location was dropped as a predictor, since location was completely confounded with the predictor mean SST (every location had a different mean SST; Table 1). Furthermore, sequencing depth was dropped from the OTU analysis since it was not expected to influence the presence or absence of a species. Details for Boral model-fitting procedure can be found in the Supplement (Details of model fitting procedure in Boral and additional analysis), including residual analysis (Fig. S3), parameter estimates (Fig. S4), and a supplementary analysis confirming the insignificance of sequencing depth (Fig. S5). Similarly to the GDM, the analysis was also run for the smaller sample set (47 samples) also including the predictors host polyp size, bleaching status, and *in situ* temperature for evaluating the effect of more host characteristics on residual correlations (Fig. S6).

For the primary OTU-based analysis, the relative influences of the predictors and latent variables were inferred by fitting a series of single-factor latent variable models, one for each environmental predictor as in Letten et al. (2015). We used the default priors available in 'boral' for Bayesian Markov chain Monte

Carlo (MCMC) parameter estimations. For both the OTUs and MED nodes, estimated correlations due to shared environmental responses and due to latent variables were illustrated as correlation plots. A significant correlation was declared as one whose corresponding 95% credible interval did not contain zero. Estimated parameter coefficients of the complete model were visualized using caterpillar plots of the estimated posterior median and the corresponding 95% credible interval (Fig. S4). The R scripts used for 'boral' and GDM analysis may be retrieved from www.github.com/patrichiahimeka/coralSymbionts.

3. RESULTS

The OTU clustering approach based on 97% identity determined 18 Symbiodiniaceae OTUs after filtering (Fig. 2a, Table S1). The most abundant OTUs were identified as ITS2 types C1, C21a, and D1; also common were C3, C3b, D2, C21/3d/C3k and types closely related to C21/3d/C3k. The MED pipeline distinguished 235 nodes that mapped to 24 different Symbiodiniaceae ITS2 types (Fig. 2b, Table S2). The majority of nodes (148) and 63% of all sequences (total N = 3.86 M) belonged either to C1, D1, or C21a. The numerous C1-related types were mostly present at background levels and consisted of a mix of well-known and rare types: C1b, C1c, C1h, and C1p, which have wide geographic distributions and are dominant in other coral taxa (Franklin et al. 2012), and C1005, C1013, C1060, and C1085, which have only been reported once and in other host species (Reimer et al. 2006). Some types were both rare and previously unknown from Japan, such as C1148 from Western Australia, C1234 from the South China Sea, and C1002 from the Caribbean (Garren et al. 2006), and some were found only as intragenomic variants of C1 (C1226, C1228, C1230) (Thornhill et al. 2007). Most ITS2 types previously reported for *G. fascicularis* were confirmed, except for C3u from the Indian Ocean (Franklin et al. 2012) and C2r, D17, and C161 from Hainan Island (Zhou et al. 2017). Many of the less abundant ITS2 types found by the MED were not detected with the OTU approach, and some of the ITS2 types were only identified by the OTU approach but not by MED, such as C1008, C90, C91, C15, and one OTU distantly related to C22 (96%).

Symbiodiniaceae communities clustered into 3 main community types, each characterized by the dominance of ITS2 types C1, C21a, or D1, except for 3 samples that were mostly inhabited by C3 or C27 (Figs. 2 & 3). The community types each associated with a different

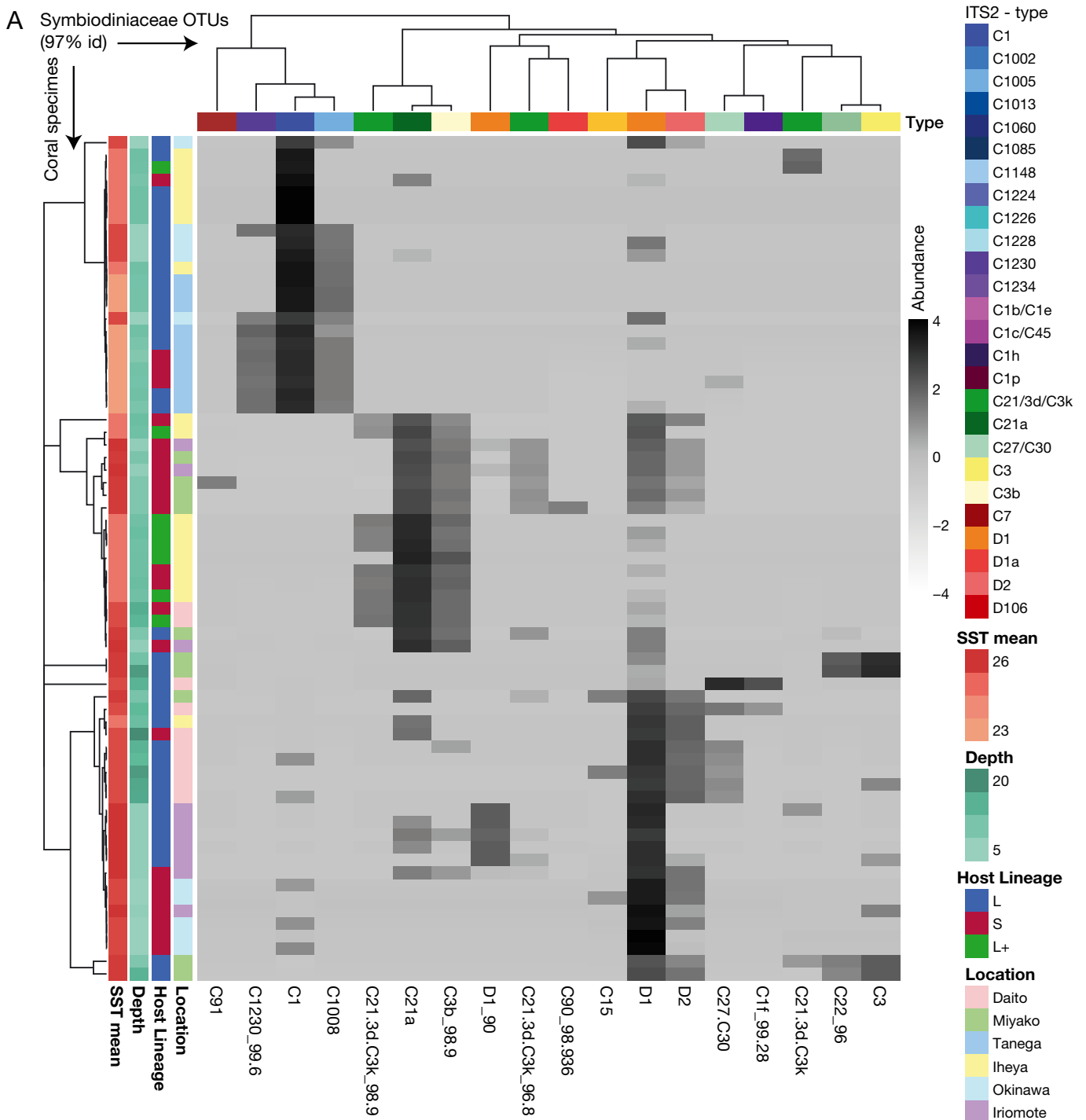
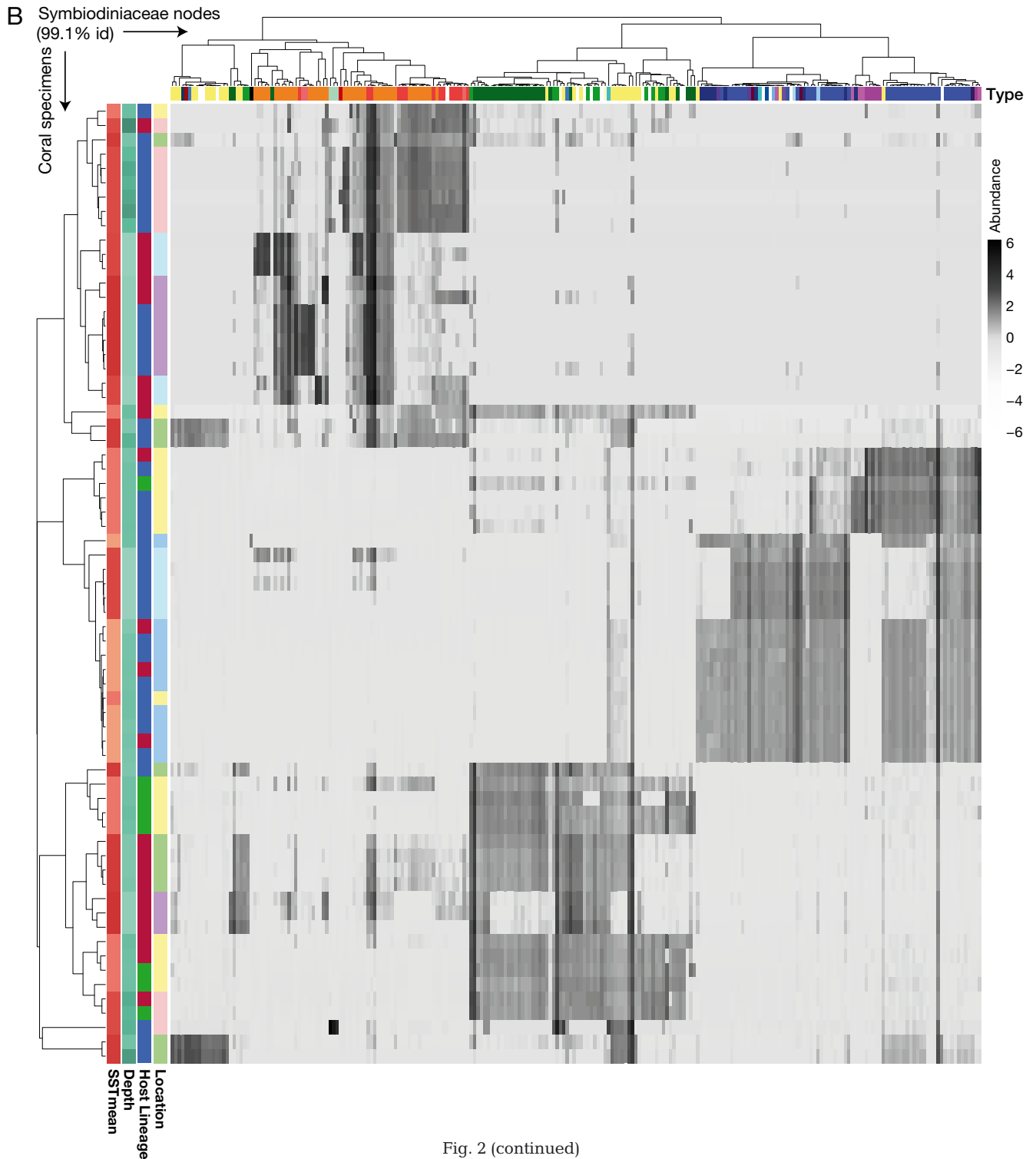


Fig. 2. Abundances and co-occurrences of Symbiodiniaceae ITS2 types (columns) across 67 *Galaxea fascicularis* colonies (rows). The colors at the top of the heatmaps indicate the closest matching ITS2 type of the (A) operational taxonomic units (OTUs) and (B) MED-nodes; uncolored MED-nodes in (B) did not match to any of the previously known ITS2-types (GenBank accession numbers in Tables S1 & S2); communities are colored according to their source locations, annual mean sea surface temperature (SST, °C), and depth (m). (A) 18 OTUs inferred with within-sample clustering to 97% sequence identity. Numbers in the OTU labels separated with an underscore refer to percentage sequence identity of an OTU to the indicated ITS2 type; (B) 235 nodes inferred with minimum entropy decomposition. Raw abundances were standardized across rows and log₁₀-transformed for visualization of rare OTUs. Symbiodiniaceae clustered into discrete communities that are characterized by the dominance of ITS2 types C1, D1, or C21a and each community type associated with a different collection of background ITS2 types. OTUs and communities were hierarchically clustered based on correlation and Bray-Curtis distance, respectively

Figure continued on next page



group of background ITS2 types and occurred at different frequencies among locations. For example, C1-dominated communities were the most common community type in Northern Tanegashima but were absent in Miyako, Iriomote, and Daito Islands; D1-dominated communities were the most common in Daito and Iri-

omote. Using the MED approach, we also detected some variation within community types between locations; for example, different C1-nodes were present in Iheya, Tanegashima, and Okinawa, and different D1-nodes were present in Daito, Iriomote, and Okinawa (Fig. 2). D1-dominated communities were found both

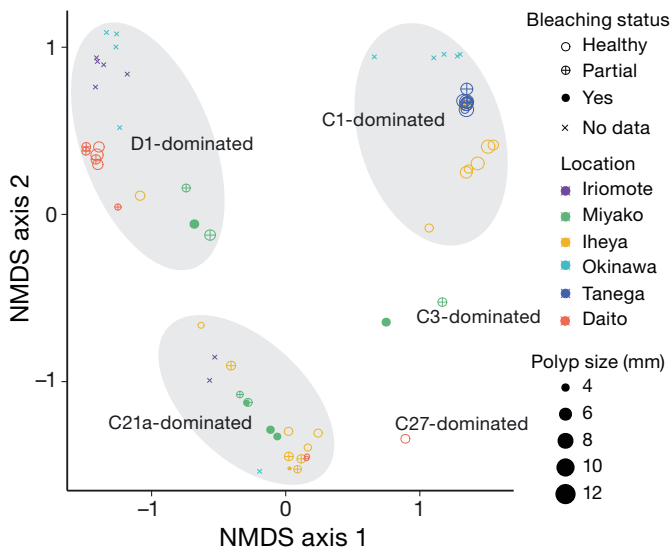


Fig. 3. Non-metric multidimensional scaling of Symbiodiniaceae community composition based on Bray-Curtis dissimilarity ($k = 2$, Stress1 = 0.087). Community types occur across multiple locations and vary in polyp size (symbol size) and bleaching status (symbol fill) of the associated hosts. Samples colored according to sampling location. Host characteristics are only depicted for specimens with available host morphological data (Table 1). Grey ovals encircle members of the same community type (D1-dominated, C1-dominated, C21a-dominated)

at the shallowest and deepest sampling sites (Fig. 3). Host characteristics co-varied with community type, in that larger polyps (2 mm larger on average) and less bleaching were associated with C1-dominated communities (Fig. 3).

The GDM over all samples identified host lineage and geographic distance as the only significant factor for explaining community composition dissimilarity (total deviance explained: 11.89%; Table 2). The analysis explained markedly less of the total variation in community dissimilarity than the second analysis on the subset of samples including more predictors, which identified polyp size as the most influential significant factor, followed by water depth, host lineage, geographic distance, and sequencing depth (total explained deviance = 29.29%; Table 2). Temperature variables in both analyses explained the most deviance relative to the other predictors but were not significant based on the permutation test. Running the model with other SST parameters (annual minima, maxima, means of collection months, annual variation) did not change this result (data not shown). This could be due to the

fact that temperature values were only available at the site level and 1 of 3 or 4 values corresponding to the broader geographic region (Tanegashima, Okinawa, Daito, Iriomote).

The joint species distribution model applied to the 97%-clustered OTUs revealed that the co-occurrence of Symbiodiniaceae could be explained partially by host and environmental predictors, but that there was still considerable residual covariation accounted for by the inclusion of latent variables (Table 3, Fig. 4; parameter coefficients in Fig. S4). The influence of predictors estimated in the set of single factor models varied in their fit to the OTUs (Table 3). On average, the annual mean SST of a sampling location explained the distribution of the OTUs best (5–97%, on average 48%), followed by host lineage (4–80%, on average 29%), and finally depth (2–62%, on average 18%). The OTUs clustered into 2 arbitrary groups of positive pairwise correlations (marked in blue, Fig. 4a, left) due to environmental and host similarity, one including D1, D2, and C3, and a second group containing C1-related types. The residual correlations explained by the latent variables were noticeably stronger in magnitude and split the OTUs roughly into 3 groups (Fig. 4a, right). The analysis of MED nodes exhibited high correlations within groups of nodes, forming distinct clusters especially in the residual correlation explained by the latent variables (Fig. 4b, right). From both the estimated environmental and latent correlations, there is evidence that C21a and C21-related types (*Cladocopium*) and D-types (*Durusdinium*) more often co-occur together than either of them do with C1 and C1-related types, which was consistent with the analysis considering more predictors (Fig. S6).

Table 2. Relative importance (Rel. imp.) and significance of environmental variables for Symbiodiniaceae community composition from generalized dissimilarity modeling (GDM). Done once for all 67 samples and once for 47 samples including all metadata. SST: sea surface temperature; NA: not available; dropped: variables that were dropped by GDM during model selection

Predictors	67 samples		47 samples	
	Rel. imp.	p	Rel. imp.	p
Bleaching status	NA		0.56	0.43
Polyp size	NA		12.93	<0.001
Temperature <i>in situ</i>	NA		0.76	0.33
SST mean	17.53	0.15	10.46	0.12
Depth	(Dropped)		8.66	<0.001
Host genotype (PC1)	16.53	<0.001	6.88	<0.001
Host genotype (PC2)	0.53	0.71	0.00	0.78
Geographic distance	11.03	0.01	1.94	<0.001
Total deviance explained	11.94 %		28.78 %	
Model deviance	1167.64	0.01	460.79	<0.001

Table 3. Proportion of variation explained by predictors and latent variables based on operational taxonomic units (OTUs) clustered to 97% identity as inferred by the joint species distribution model. Summarizes the proportions explained by the total of predictors and latent variables (LV) in the main analysis (full model, 2nd column), and the proportions explained by each predictor relative to the latent variables (single factor models, 3rd to 5th columns: SST, sea surface temperature; Host lin., host lineage) as fitted in each a separate single factor model

OTUs	Full model Predictors vs. LV	Single factor models		
		SST	Host lin.	Depth
C1		0.58	0.04	0.04
C1008		0.41	0.14	0.14
C1230_99.6		0.26	0.09	0.07
C15		0.46	0.34	0.10
C1f_99.3		0.28	0.52	0.34
C21.3d.C3k		0.36	0.80	0.52
C21.3d.C3k_98.9		0.07	0.38	0.15
C21.3d.C3k_96.8		0.85	0.12	0.13
C21a		0.12	0.11	0.02
C22_96		0.84	0.51	0.13
C27.C30		0.05	0.38	0.44
C3		0.82	0.22	0.05
C3b_98.9		0.14	0.19	0.03
D1		0.74	0.12	0.11
D1_90		0.97	0.30	0.62
D2		0.68	0.41	0.03

4. DISCUSSION

Our results illuminate structure and assembly of coral-associated Symbiodiniaceae using the example of the reef-building coral *Galaxea fascicularis*. We finely resolved the genetic variation of ITS2 sequences between coral samples and tested for effects of environmental variation and host characteristics on Symbiodiniaceae community composition using GDM. Further, we used latent variable modeling to infer pairwise correlations among individual Symbiodiniaceae taxa that are apparently above and beyond that explained by similar environmental responses. We found 3 main community types hosted by *G. fascicularis*, characterized by the dominance of the ITS2 types C1 and C21a of the genus *Cladocopium*, or D1 of the genus *Durusdinium*, each of which was associated with a different group of background types and/or intragenomic variants. Overall, host factors and environmental conditions accounted for a large portion of variation in the co-occurrences of these Symbiodiniaceae taxa, but the latent variables representing processes residual to this were also important

in structuring the communities and is a possible signature of biotic interactions.

4.1. Genetic background variation within communities and ITS-types

In general, the MED analysis identified much more diversity of ITS2 sequences in *G. fascicularis* than the 97% OTU clustering (Fig. 2). However, most MED nodes grouped into several regular, co-varying clusters of nearly perfect correlations, which are likely to represent intra-genomic ITS2 variants within a single Symbiodiniaceae taxon. These clusters persisted even after controlling for exogenous factors (Fig. 4b), further supporting this hypothesis. Usually, a cluster consisted of more than one ITS2 type, illustrating how a single genome may contain several different ITS2 types. At the same time, ITS2 types and even individual nodes were often represented in more than one cluster. For example, some of the C3 and C21a nodes formed clusters of perfect correlation, while other C3-nodes almost exclusively occurred in certain samples from Miyako Island, in which they were also dominant.

This underscores the challenges involved in using ITS2 as a descriptor of Symbiodiniaceae diversity, sometimes over-splitting intragenomic variants and sometimes over-aggregating ecologically distinct taxa (LaJeunesse & Thornhill 2011, Reimer et al. 2017). In the case of C21a and C3, it was shown that C21a is derived from the ancestral sequence C3 (Correa & Baker 2009) and occurs in the same ribosomal repeat array (LaJeunesse et al. 2004). It is likely that similar processes are responsible for the diversity patterns also present in the C1-related and ITS2 types of the genus *Durusdinium* (clade D). Nevertheless, the use of an additional genetic marker such as the *psbA* region (as in LaJeunesse & Thornhill 2011, Smith et al. 2017) seems inevitable for clearly distinguishing intragenomic variation from species diversity.

Although this was not an *a priori* goal of our analysis, the joint species distribution model is a potentially useful tool for separating intragenomic variants versus lineage-level variation from the correlation structure among types. Even if the genetic boundaries between individual taxa within a community were unclear, the fine resolution of the MED ap-

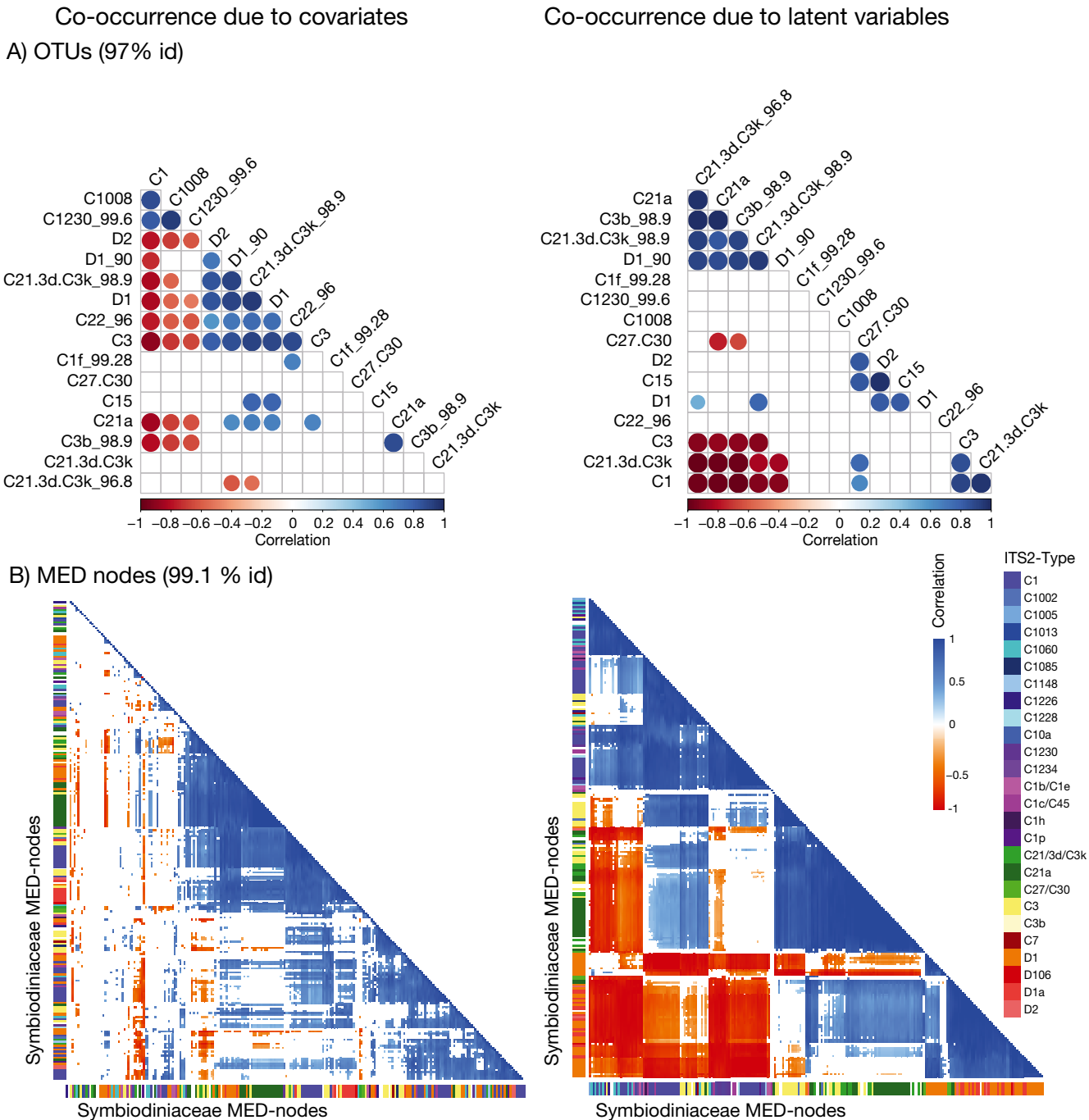


Fig. 4. Estimated pattern of correlations of Symbiodiniaceae ITS2 types explained by the covariates mean sea surface temperature, water depth, and host lineage (left) or by latent variables (right) as inferred by joint species distribution modeling. (A) Analysis of 97%-clustered operational taxonomic units (OTUs) (Fig. 2A), (B) analysis of 99.11% identical minimum entropy decomposition (MED) nodes (Fig. 2B). In (B), the axes are colored to represent the closest matching ITS2 type of the 235 MED nodes. Strong correlations (blue: positive; red: negative) exist even after controlling for environmental factors, which may indicate interspecific interactions. OTUs and MED nodes are sorted by hierarchical clustering. Numbers in the OTU labels separated with an underscore refer to percentage sequence identity of an OTU to the indicated ITS2 type

proach still gave insight into how the ITS2 diversity as a whole changed from community to community. By interpreting the correlation patterns of finely resolved ITS2 sequences across communities and geo-

graphic locations, the nature of ITS2 sequence assembly within genomes or communities may be distinguished, similar to what has been suggested by the metahaplotype concept (Smith et al. 2017). For

example, the correlation patterns of MED nodes revealed that Symbiodiniaceae species (ITS2 types) may have undergone spatially driven genetic differentiation, since varying sets of MED nodes of the same ITS2 type vary between locations (e.g. in C1 between Tanegashima, Okinawa and Iheya; in D1 between Daito, Okinawa and Iriomote; Fig. 2b). This would not have been detected by the coarser OTU clustering approach, since this variation is given by nodes that were genetically more similar than 97% of the same or closely related ITS2 types. The MED approach may thus also be useful in inferring spatial differentiation patterns within Symbiodiniaceae taxa as an alternative to population genetic tools such as microsatellite markers.

4.2. Environment and geography

The environment and geographic space have long been known to have a large influence on the distribution of Symbiodiniaceae (Baker 2003). Accordingly, we confirmed an effect of geographic location and distance for Symbiodiniaceae inhabiting *Galaxea fascicularis* (Table 2), as already known from previous reports across its distribution range (Franklin et al. 2012, Zhou et al. 2017). The 3 Symbiodiniaceae community types found here—C1-dominated, C21a-dominated, and D1-dominated—varied in their frequencies across the study area, and the genetic compositions within ITS2 types (MED nodes) differed between sampling locations (Figs. 2 & 3a). The location-specific differences within ITS2 types suggests that neutral, spatially driven processes such as drift could influence the differentiation of ITS2 types and their community structure. Spatial differentiation on a regional scale in Symbiodiniaceae has been shown in population genetic studies with microsatellite markers (Howells et al. 2013, Baums et al. 2014), perhaps indicating dispersal limitations of Symbiodiniaceae in their free-living stage. In future studies, the precise location of each colony within a reef should also be monitored, in order to account for the potential influence of spatial distances between colonies on the local distribution of Symbiodiniaceae communities.

Temperature was highly correlated with geographic location in our study. In the joint species distribution model, where location had been dropped as a predictor due to co-variation with annual mean SST, mean SST explained the distribution of individual Symbiodiniaceae taxa well (Table 3), confirming the importance of temperature shown in numerous biogeographic field studies (e.g. Noda et al. 2017) and

laboratory experiments (Rowan 2004, Sampayo et al. 2008), including in our study taxon (Chen et al. 2005, Tong et al. 2017, Zhou et al. 2017). Unexpectedly, temperature was not significant in the GDM analysis of community composition (Table 2), even when tested for any other temperature metric (min. SST, max. SST, annual variation, mean of sample collection months), suggesting that when corrected for sampling location, temperature may not be as influential for Symbiodiniaceae community composition in *Galaxea* as previously thought (Chen et al. 2005, Tong et al. 2017, Zhou et al. 2017). *Galaxea* was shown to be relatively resistant to elevated sea temperatures (Marshall & Baird 2000), and a recent study found no seasonal variation in *Galaxea*-hosted Symbiodiniaceae communities in Hong Kong, where SST annually ranges from 14–31°C (Cai et al. 2018). However, it is possible that fine-scale differences in heat distribution within the reef are responsible for Symbiodiniaceae composition rather than latitudinal temperature gradients averaged over a sampling site. Temperature was recorded here at the level of the sampling site, but the highest variation in community dissimilarities existed within sites (i.e. in Iheya, all main community types—C1-, C21a-, and D1-dominated—were found at all depths; Figs. 2 & 3), which may explain the non-significance of temperature for community composition across the archipelago in our study.

Water depth is one of the factors that alters heat distribution within a reef. Depth was a significant predictor of community composition and the distribution of individual taxa (Tables 2 & 3), which is in line with the zonation of Symbiodiniaceae ITS2 types along depth gradients observed in many coral species (Bongaerts et al. 2015). Similar to previous reports, we found D1-dominated communities to have the widest depth range (Fig. 2). The genus *Durusdinium* (clade D) often occurs in higher abundances at the shallowest and deepest habitats within the ideal coral habitat range (Toller et al. 2001, Baker 2003), which could be explained by the fact that *Durusdinium* is able to tolerate higher temperature ranges than other genera (Cooper et al. 2011). Thus, although temperature could not be directly measured at the colony-level, it may have had a hidden effect on community composition via the factor depth.

Other unmeasured factors correlating with water depth or temperature could explain the effect on the distribution of Symbiodiniaceae physiologically. Nutrient levels change with water depth (Parsons et al. 2013), and a higher nutrient range was shown to be tolerated by the genus *Durusdinium* (Cooper et al. 2011). Light levels also rapidly decrease with depth

and differentially affect the growth optima of Symbiodiniaceae types (Klueter et al. 2017). Other microhabitat differences such as water movement (Monismith 2007), substrate (Cunning et al. 2015), or syntopic fauna (Mihaljevic 2012) may additionally influence the distribution of Symbiodiniaceae within a reef, and should therefore be monitored carefully in future studies.

4.3. Host characteristics

Host characteristics had larger influences on Symbiodiniaceae community composition than environmental predictors, and the model including more host characteristics was a better fit to community composition in *G. fascicularis* than the model without (Table 2). This finding may be driven by the fact that C1-dominated Symbiodiniaceae communities were associated with the largest polyp sizes and often with the host genetic lineage 'L', whereas C21a-dominated communities were associated with smaller polyps and mostly with host lineages 'S' and 'L+' (Figs. 2 & 3). The ITS2 type C1 has been linked to faster skeletal growth (Jones & Berkelmans 2010), higher efficiency in carbon (Little et al. 2004) and nitrogen (Baker et al. 2013) transfer rates to the host than types of *Durisdinium* under normal environmental conditions, which could explain the association of C1 communities with larger polyps in our study. Not much is known about how skeletal growth rate affects polyp size in colonies directly, but an early study in *Acropora* branches identified by far the highest calcification rates in the terminal enlarged polyps of a branch (Goreau & Goreau 1959). In contrast, larger polyps correlated negatively with growth rates in the solitary coral *Balanophyllia* (Goffredo et al. 2008). Computer tomographic analyses of the skeleton are needed to determine growth rate in *G. fascicularis*, which was beyond the scope of our study.

We found that large physiological differences also potentially exist between ITS-types of the same genus, i.e. in *Cladocopium* between C1 and C21a. C21a may be physiologically closer to *Durisdinium* type D1 than C1, as indicated by their positive correlations in the joint species distribution model (Fig. 4), especially if more host factors are incorporated (Fig. S6). C21a and D1 were shown to have similar and higher thermotolerance levels than C1 (Swain et al. 2017), which could also explain their more frequent association with *Galaxea* colonies with more bleaching (Fig. 3). C21a- (and C3)-dominated communities especially were often associated with par-

tially or fully bleached corals. Although not significant in the GDM analysis, the effect of bleaching status should be further investigated in future studies with better sampling photographs, using a flash and/or *in situ* fluorescence measurements.

Host lineage had a large influence on community composition, particularly if other host characteristics were not considered (Table 2). This implies that generalist host species may not be as 'general' in their symbiotic associations. Across our study sites, most C21a-dominated communities associated with lineages 'S' and 'L+', C1-dominated communities associated with lineage 'L', and D1-dominated communities were mixed (Fig. 3). Differences in Symbiodiniaceae composition between cryptic species have been shown in other corals (Prada et al. 2014, Warner et al. 2015), emphasizing the importance of correct species delimitations of both symbiotic partners (Parkinson & Baums 2014). Despite some indications for functional differentiation between the genetic lineages in *G. fascicularis* (Watanabe et al. 2005, Wewengkang et al. 2007) this matter is still unresolved, and yet undetected variation in functional traits in the host may be related to differential symbiont or host selection. However, host lineage also correlated with polyp size, and the causal relationships between these factors and to Symbiodiniaceae community composition remain to be resolved in future studies. Sequencing depth only had a minor influence on community composition (Table 2), indicating that community compositions were not confounded by sequencing-related sampling biases.

Our study did not investigate variation in community composition within the host colony. Within-colony variation according to varying light levels across a colony has been found in other coral species (van Oppen et al. 2001, Stat et al. 2011) and could potentially confound our results regarding the role of exogenous factors for Symbiodiniaceae community composition. However, there is evidence that the distribution of Symbiodiniaceae in *Galaxea* could be quite uniform, since the *G. fascicularis* colonies sampled were planar with little chance for self-shading and therefore would have experienced similar light levels across the colony. Further, 2 clonal colonies (PW112 and PW117) probably emerged by fragmentation (determined by RAD-seq; Wepfer 2018) and also hosted the same Symbiodiniaceae community, suggesting that once established, communities are stable. Whether or not there is variation within the colony, within-colony variation would only decrease the predictive power of the exogenous factors for community composition. We found that the exoge-

nous factors only partially explained the distribution of Symbiodiniaceae, implying that community composition within individual polyps could be shaped by random processes, intragenomic variation, or perhaps intrinsic biotic interactions, depending on the level of resolution of the OTUs analyzed.

4.4. Co-occurrences between Symbiodiniaceae OTUs

The communities studied generally clustered in to 3 groups (Fig. 2), which is in line with the regularity of Symbiodiniaceae communities documented across a phylogenetically diverse number of coral hosts (Ziegler et al. 2018). Using joint species distribution modeling, we further found that this regularity may only be partly related to underlying variation in the physical or host environment, and that such correlation patterns may still persist in the residual component of the model explained by the latent variables (Tables 2 & 3, Fig. 4). This implies that other underlying causes are likely responsible for the co-occurrences of certain Symbiodiniaceae taxa. These causes may represent undetected environmental predictors relating to microhabitat differences as mentioned above, unmeasured host characteristics like varying life histories, or possibly metabolic or behavioral interactions between Symbiodiniaceae taxa (separated by more than 3% genetic differentiation to eliminate the potential for intragenomic variation).

Generally, more frequent co-occurrences were found between phylogenetically more similar ITS2 types, for example C21a with C21/3d/3k, D1 with D2, and C1 with other C1-related types. In contrast, the dominant OTUs C1, C21a, and D1 tended to exclude each other in the endosymbiotic community, even after controlling for environmental factors like temperature, known to influence their competitive success (Baker et al. 2013). One explanation for this could be shared host or environmental preferences among closely related symbionts that were not captured in our predictors.

Of the 3 dominant taxa, C21a may be more tolerant to phylogenetically distant types, as indicated by the positive correlation to D1 (Fig. 4). Similarly, a recent study by Zhou et al. (2017) found C21a to occur with more different types in higher percentages than the other dominant lineages, suggesting that C21a may generally be found in phylogenetically more diverse communities. The higher association of C21a-dominated communities with partially or fully bleached corals in this study (Fig. 3) could indicate that this is a

transitional community capable of persisting in stress situations. A more diverse community would be better able to accommodate stressful conditions across different ecosystems (Ives & Carpenter 2007), which was recently demonstrated numerically for Symbiodiniaceae (Ziegler et al. 2018). A similar conclusion may be made for the 2 C3-dominated communities in our study (Figs. 2 & 3); however these were much less common, and more observations are needed. To test a potentially higher diversity in C21a and C3-dominated communities during stress, future research should conduct controlled bleaching experiments with *Galaxea* colonies hosting predominantly C21a or C3 vs. D1 or C1.

The approach to analyzing community assembly in Symbiodiniaceae by joint species distribution modeling could integrate functional species traits as a next step to investigate potential interspecific interactions (McGill et al. 2006, Warton et al. 2015b). Comprehensive characterizations of Symbiodiniaceae taxa including, for example, comparisons of thermotolerance and photoacclimation (Diaz-Almeyda et al. 2017, Swain et al. 2017) are increasing and will markedly advance this field. Lastly, experimental manipulations between different Symbiodiniaceae taxa in communities is a technically challenging but potentially definitive route to explaining the mechanisms behind statistically detectable patterns of community structure.

5. CONCLUSIONS

We conclude that Symbiodiniaceae *in hospite* assemble into regularly structured communities. Based on the high resolution of diversity patterns obtained by the MED approach, we were able to distinguish local varieties of the same ITS2 type. The community structure was partially explained by environmental variables such as location, temperature, and depth, but also host characteristics such as polyp size and genetic lineage, confirming the importance of precise species identification of both symbiotic partners. After accounting for exogenous factors, a latent structure of co-occurrences between Symbiodiniaceae taxa emerged, perhaps indicating the existence of positive and negative interactions between sets of Symbiodiniaceae taxa. We have much to learn about the ecology of the coral symbiosis, but understanding the assembly of Symbiodiniaceae communities will help us understand the resilience of corals and the ecosystems they support to ongoing climate change.

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LITERATURE CITED

- ✦ Arif C, Daniels C, Bayer T, Banguera-Hinestroza E and others (2014) Assessing *Symbiodinium* diversity in scleractinian corals via next-generation sequencing-based genotyping of the ITS2 rDNA region. *Mol Ecol* 23: 4418–4433
- ✦ Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. *Ann Rev Ecol Evol Systematics* 40:551–571
- ✦ Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Annu Rev Ecol Evol Syst* 34: 661–689
- ✦ Baker DM, Andras JP, Jordan-Garza AG, Fogel ML (2013) Nitrate competition in a coral symbiosis varies with temperature among *Symbiodinium* clades. *ISME J* 7:1248–1251
- ✦ Baums IB, Devlin-Durante MK, Lajeunesse TC (2014) New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies. *Mol Ecol* 23:4203–4215
- Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proc R Soc B* 273:2305–2312
- Björk JR, Hui FK, O'Hara RB, Montoya JM (2018) Uncovering the drivers of host associated microbiota with joint species distribution modeling. *Mol Ecol* 27:2714–2724
- ✦ Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120
- ✦ Bongaerts P, Carmichael M, Hay KB, Tonk L, Frade PR, Hoegh-Guldberg O (2015) Prevalent endosymbiont zonation shapes the depth distributions of scleractinian coral species. *R Soc Open Sci* 2:140297
- ✦ Boucher DH, James S, Keeler KH (1982) The ecology of mutualism. *Annu Rev Ecol Syst* 13:315–347
- ✦ Boulotte NM, Dalton SJ, Carroll AG, Harrison PL, Putnam HM, Peplow LM, van Oppen MJH (2016) Exploring the *Symbiodinium* rare biosphere provides evidence for symbiont switching in reef-building corals. *ISME J* 10: 2693–2701
- ✦ Cai L, Zhou G, Tong H, Tian RM and others (2018) Season structures prokaryotic partners but not algal symbionts in subtropical hard corals. *Appl Microbiol Biotechnol* 102: 4963–4973
- ✦ Callahan BJ, McMurdie PJ, Holmes SP (2017) Exact sequence variants should replace operational taxonomic units in marker gene data analysis. *ISME J* 11:2639–2643
- ✦ Chen CA, Wang JT, Fang LS, Yang YW (2005) Fluctuating algal symbiont communities in *Acropora palifera* (Scleractinia: Acroporidae) from Taiwan. *Mar Ecol Prog Ser* 295:113–121
- ✦ Chesson P (2000) Mechanisms of maintenance of species diversity. *Annu Rev Ecol Syst* 31:343–366
- ✦ Cooper TF, Berkelmans R, Ulstrup KE, Weeks S and others (2011) Environmental factors controlling the distribution of *Symbiodinium* harboured by the coral *Acropora millepora* on the Great Barrier Reef. *PLOS ONE* 6:e25536
- ✦ Correa AMS, Baker AC (2009) Understanding diversity in coral-algal symbiosis: a cluster-based approach to interpreting fine-scale genetic variation in the genus *Symbiodinium*. *Coral Reefs* 28:81–93
- ✦ Cunning R, Yost DM, Guarinello ML, Putnam HM, Gates RD (2015) Variability of *Symbiodinium* communities in waters, sediments, and corals of thermally distinct reef pools in American Samoa. *PLOS ONE* 10:e0145099
- ✦ Cunning R, Gates RD, Edmunds PJ (2017) Using high-throughput sequencing of ITS2 to describe *Symbiodinium* metacommunities in St. John, US Virgin Islands. *PeerJ* 5:e3472
- Diaz-Almeyda EM, Prada C, Ohdera AH, Moran H and others (2017) Intraspecific and interspecific variation in thermotolerance and photoacclimation in *Symbiodinium* dinoflagellates. *Proc R Soc B* 284:20171767
- ✦ Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 10:996–998
- ✦ Eren AM, Morrison HG, Lescault PJ, Reveillaud J, Vineis JH, Sogin ML (2015) Minimum entropy decomposition: unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. *ISME J* 9:968–979
- ✦ Ferrier S, Manion G, Elith J, Richardson K (2007) Using generalized dissimilarity modelling to analyse and predict patterns of beta diversity in regional biodiversity assessment. *Divers Distrib* 13:252–264
- ✦ Franklin EC, Stat M, Pochon X, Putnam HM, Gates RD (2012) GeoSymbio: a hybrid, cloud-based web application of global geospatial bioinformatics and ecoinformatics for *Symbiodinium*-host symbioses. *Mol Ecol Resour* 12:369–373
- ✦ Garren M, Walsh SM, Caccone A, Knowlton N (2006) Patterns of association between *Symbiodinium* and members of the *Montastraea annularis* species complex on spatial scales ranging from within colonies to between geographic regions. *Coral Reefs* 25:503–512
- ✦ Goffredo S, Caroselli E, Mattioli G, Pignotti E, Zaccanti F (2008) Relationships between growth, population structure and sea surface temperature in the temperate solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). *Coral Reefs* 27:623–632
- ✦ Goreau TF, Goreau NI (1959) The physiology of skeleton formation in corals. 2. Calcium deposition by hermatypic corals under various conditions in the reef. *Biol Bull (Woods Hole)* 117:239–250
- ✦ Gorospe KD, Donahue MJ, Karl SA (2015) The importance of sampling design: spatial patterns and clonality in estimating the genetic diversity of coral reefs. *Mar Biol* 162: 917–928
- ✦ Howells EJ, Willis BL, Bay LK, van Oppen MJH (2013) Spatial and temporal genetic structure of *Symbiodinium* populations within a common reef-building coral on the Great Barrier Reef. *Mol Ecol* 22:3693–3708
- ✦ Hughes TP, Kerry JT, Alvarez-Noriega M, Alvarez-Romero JG and others (2017) Global warming and recurrent mass bleaching of corals. *Nature* 543:373–377
- ✦ Hui FK (2016) BORAL—Bayesian ordination and regression analysis of multivariate abundance data in R. *Methods Ecol Evol* 7:744–750
- ✦ Ives AR, Carpenter SR (2007) Stability and diversity of ecosystems. *Science* 317:58–62

- 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
- Klueter A, Trapani J, Archer FI, McIlroy SE, Coffroth MA (2017) Comparative growth rates of cultured marine dinoflagellates in the genus *Symbiodinium* and the effects of temperature and light. *PLOS ONE* 12:e0187707
- Kolde R (2015) pheatmap: pretty heatmaps. R package version 1.0.8.
- 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. *J Phycol* 37:866–880
- LaJeunesse TC (2005) 'Species' radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. *Mol Biol Evol* 22:570–581
- LaJeunesse TC, Thornhill DJ (2011) Improved resolution of reef-coral endosymbiont (*Symbiodinium*) species diversity, ecology, and evolution through *psbA* non-coding region genotyping. *PLOS ONE* 6:e29013
- 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. *Limnol Oceanogr* 48:2046–2054
- LaJeunesse TC, Bhagooli R, Hidaka M, deVantier L and others (2004) Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. *Mar Ecol Prog Ser* 284:147–161
- LaJeunesse TC, Parkinson JE, Reimer JD (2012) A genetics-based description of *Symbiodinium minutum* sp. nov. and *S. psygmophilum* sp. nov. (Dinophyceae), two dinoflagellates symbiotic with cnidaria. *J Phycol* 48:1380–1391
- 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
- LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr Biol* 28:2570–2580
- Lee MJ, Jeong HJ, Jang SH, Lee SY and others (2016) Most low-abundance 'background' *Symbiodinium* spp. are transitory and have minimal functional significance for symbiotic corals. *Microb Ecol* 71:771–783
- Leibold MA, Holyoak M, Mouquet N, Amarasekare P and others (2004) The metacommunity concept: a framework for multi-scale community ecology. *Ecol Lett* 7:601–613
- Letten AD, Keith DA, Tozer MG, Hui FKC (2015) Fine-scale hydrological niche differentiation through the lens of multi-species co-occurrence models. *J Ecol* 103:1264–1275
- Little AF, van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. *Science* 304:1492–1494
- Locarnini R, Mishonov A, Antonov J, Boyer T and others (2013) World ocean atlas 2013. NOAA Atlas NESDIS 73, Vol 1: temperature. National Oceanographic Data Center, Silver Spring, MD
- Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. *Coral Reefs* 19:155–163
- McGill BJ, Enquist BJ, Weiher E, Westoby M (2006) Rebuilding community ecology from functional traits. *Trends Ecol Evol* 21:178–185
- 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
- Mihaljevic JR (2012) Linking metacommunity theory and symbiont evolutionary ecology. *Trends Ecol Evol* 27:323–329
- Monismith SG (2007) Hydrodynamics of coral reefs. *Annu Rev Fluid Mech* 39:37–55
- Muscatine L, Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *Bioscience* 27:454–460
- Nakajima Y, Shinzato C, Satoh N, Mitarai S (2015) Novel polymorphic microsatellite markers reveal genetic differentiation between two sympatric types of *Galaxea fascicularis*. *PLOS ONE* 10:e0130176
- Nakajima Y, Zayasu Y, Shinzato C, Satoh N, Mitarai S (2016) Genetic differentiation and connectivity of morphological types of the broadcast-spawning coral *Galaxea fascicularis* in the Nansei Islands, Japan. *Ecol Evol* 6:1457–1469
- Noda H, Parkinson JE, Yang SY, Reimer JD (2017) A preliminary survey of zoantharian endosymbionts shows high genetic variation over small geographic scales on Okinawa-Jima Island, Japan. *PeerJ* 5:e3740
- Oksanen J, Blanchet FG, Kindt R, Legendre P and others (2012) vegan: community ecology package. R package version 2.0-5. <http://cran.r-project.org/web/packages/vegan/index.html>
- Ovaskainen O, Tikhonov G, Norberg A, Guillaume Blanchet F and others (2017) How to make more out of community data? A conceptual framework and its implementation as models and software. *Ecol Lett* 20:561–576
- Parkinson JE, Baums IB (2014) The extended phenotypes of marine symbioses: ecological and evolutionary consequences of intraspecific genetic diversity in coral-algal associations. *Front Microbiol* 5:445
- Parkinson JE, Coffroth MA (2015) New species of clade B *Symbiodinium* (Dinophyceae) from the greater Caribbean belong to different functional guilds: *S. aenigmaticum* sp. nov., *S. antillogorgium* sp. nov., *S. endomadraxis* sp. nov., and *S. pseudominutum* sp. nov. *J Phycol* 51:850–858
- Parkinson JE, Baumgarten S, Michell CT, Baums IB, LaJeunesse TC, Voolstra CR (2016) Gene expression variation resolves species and individual strains among coral-associated dinoflagellates within the genus *Symbiodinium*. *Genome Biol Evol* 8:665–680
- Parsons TR, Takahashi M, Hargrave B (2013) Biological oceanographic processes. Elsevier, Oxford
- Pinzon JH, Sampayo E, Cox E, Chauka LJ, Chen CA, Voolstra CR, LaJeunesse TC (2013) Blind to morphology: genetics identifies several widespread ecologically common species and few endemics among Indo-Pacific cauliflower corals (*Pocillopora*, Scleractinia). *J Biogeogr* 40:1595–1608
- Pochon X, Gates RD (2010) A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. *Mol Phylogenet Evol* 56:492–497
- Pochon X, LaJeunesse TC, Pawlowski J (2004) Biogeographic partitioning and host specialization among foraminiferan dinoflagellate symbionts (*Symbiodinium*; Dinophyta). *Mar Biol* 146:17–27
- Pochon X, Pawlowski J, Zaninetti, L, Rowan R (2001) High genetic diversity and relative specificity among *Symbiodinium*-like endosymbiotic dinoflagellates in soritid foraminiferans. *Mar Biol* 139:1069–1078

- Prada C, McIlroy SE, Beltran DM, Valint DJ, Ford SA, Hellberg ME, Coffroth MA (2014) Cryptic diversity hides host and habitat specialization in a gorgonian-algal symbiosis. *Mol Ecol* 23:3330–3340
- R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Reimer JD, Takishita K, Maruyama T (2006) Molecular identification of symbiotic dinoflagellates (*Symbiodinium* spp.) from *Palythoa* spp. (Anthozoa: Hexacorallia) in Japan. *Coral Reefs* 25:521–527
- Reimer JD, Herrera M, Gatins R, Roberts MB, Parkinson JE, Berumen ML (2017) Latitudinal variation in the symbiotic dinoflagellate *Symbiodinium* of the common reef zoantharian *Palythoa tuberculosa* on the Saudi Arabian coast of the Red Sea. *J Biogeogr* 44:661–673
- Roberts CM, McClean CJ, Veron JEN, Hawkins JP and others (2002) Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science* 295:1280–1284
- Rowan R (2004) Thermal adaptation in reef coral symbionts. *Nature* 430:742
- Rowan R, Powers DA (1991) Molecular genetic identification of symbiotic dinoflagellates (Zooxanthellae). *Mar Ecol Prog Ser* 71:65–73
- Sampayo EM, Franceschinis L, Hoegh-Guldberg O, Dove S (2007) Niche partitioning of closely related symbiotic dinoflagellates. *Mol Ecol* 16:3721–3733
- Sampayo EM, Ridgway T, Bongaerts P, Hoegh-Guldberg O (2008) Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *Proc Natl Acad Sci USA* 105:10444–10449
- Schindelin J, Arganda-Carreras I, Frise E, Kaynig V and others (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* 9:676–682
- Simberloff D, Dayan T (1991) The guild concept and the structure of ecological communities. *Annu Rev Ecol Syst* 22:115–143
- Smith EG, Ketchum RN, Burt JA (2017) Host specificity of *Symbiodinium* variants revealed by an ITS2 metahaplo-type approach. *ISME J* 11:1500–1503
- Stat M, Bird CE, Pochon X, Chasqui L and others (2011) Variation in *Symbiodinium* ITS2 sequence assemblages among coral colonies. *PLOS ONE* 6:e15854
- Swain TD, Chandler J, Backman V, Marcelino L (2017) Consensus thermotolerance ranking for 110 *Symbiodinium* phylotypes: an exemplar utilization of a novel iterative partial-rank aggregation tool with broad application potential. *Funct Ecol* 31:172–183
- Thornhill DJ, Lajeunesse TC, Santos SR (2007) Measuring rDNA diversity in eukaryotic microbial systems: how intragenomic variation, pseudogenes, and PCR artifacts confound biodiversity estimates. *Mol Ecol* 16:5326–5340
- Toller WW, Rowan R, Knowlton N (2001) Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. *Biol Bull (Woods Hole)* 201:348–359
- Tong H, Cai L, Zhou G, Yuan T and others (2017) Temperature shapes coral-algal symbiosis in the South China Sea. *Sci Rep* 7:40118
- van Oppen MJH, Palstra FP, Piquet AMT, Miller DJ (2001) Patterns of coral-dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. *Proc R Soc B* 268:1759–1767
- Warner PA, van Oppen MJH, Willis BL (2015) Unexpected cryptic species diversity in the widespread coral *Seriatopora hystrix* masks spatial-genetic patterns of connectivity. *Mol Ecol* 24:2993–3008
- Warton DI, Blanchet FG, O'Hara RB, Ovaskainen O, Taskinen S, Walker SC, Hui FKC (2015a) So many variables: joint modeling in community ecology. *Trends Ecol Evol* 30:766–779
- Warton DI, Foster SD, De'ath G, Stoklosa J, Dunstan PK (2015b) Model-based thinking for community ecology. *Plant Ecol* 216:669–682
- Warton DI, Blanchet FG, O'Hara R, Ovaskainen O, Taskinen S, Walker SC, Hui FK (2016) Extending joint models in community ecology: a response to Beissinger et al. *Trends Ecol Evol* 31:737–738
- Watanabe T, Nishida M, Watanabe K, Wewengkang DS, Hidaka M (2005) Polymorphism in nucleotide sequence of mitochondrial intergenic region in scleractinian coral (*Galaxea fascicularis*). *Mar Biotechnol (NY)* 7:33–39
- Wepfer PH (2018) Spatial genetic structure in the coral genus *Galaxea* (Euphyllidae) and their associated Symbiodiniaceae communities. PhD thesis, Okinawa Institute of Science and Technology
- Wewengkang DS, Watanabe T, Hidaka M (2007) Studies on morphotypes of the coral *Galaxea fascicularis* from Okinawa: polyp color, nematocyst shape, and coenosteum density. *J Japan Coral Reef Soc* 9:49–59
- Yost DM, Wang LH, Fan TY, Chen CS, Lee RW, Sogin E, Gates RD (2013) Diversity in skeletal architecture influences biological heterogeneity and *Symbiodinium* habitat in corals. *Zoology* 116:262–269
- Zhou G, Huang H, Lian J, Zhang C, Li X (2012) Habitat correlation of *Symbiodinium* diversity in two reef-building coral species in an upwelling region, eastern Hainan Island, China. *J Mar Biol Assoc UK* 92:1309–1316
- Zhou G, Cai L, Li Y, Tong H and others (2017) Temperature-driven local acclimatization of *Symbiodinium* hosted by the coral *Galaxea fascicularis* at Hainan Island, China. *Front Microbiol* 8:2487
- Ziegler M, Eguíluz VM, Duarte CM, Voolstra CR (2018) Rare symbionts may contribute to the resilience of coral-algal assemblages. *ISME J* 12:161–172

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