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Linking photoacclimation responses and microbiome shifts between depth-segregated sibling species of reef corals

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Metazoans host complex communities of microorganisms that include dinoflagellates, fungi, bacteria, archaea and viruses. Interactions among members of these complex assemblages allow hosts to adjust their physiology and metabolism to cope with environmental variation and occupy different habitats. Here, using reciprocal transplantation across depths, we studied adaptive divergence in the corals *Orbicella annularis* and *O. franksi*, two young species with contrasting vertical distribution in the Caribbean. When transplanted from deep to shallow, *O. franksi* experienced fast photoacclimation and low mortality, and maintained a consistent bacterial community. By contrast, *O. annularis* experienced high mortality and limited photoacclimation when transplanted from shallow to deep. The photophysiological collapse of *O. annularis* in the deep environment was associated with an increased microbiome variability and reduction of some bacterial taxa. Differences in the symbiotic algal community were more pronounced

between coral species than between depths. Our study suggests that these sibling species are adapted to distinctive light environments partially driven by the algae photoacclimation capacity and the microbiome robustness, highlighting the importance of niche specialization in symbiotic corals for the maintenance of species diversity. Our findings have implications for the management of these threatened Caribbean corals and the effectiveness of coral reef restoration efforts.

1. Introduction

Understanding how microbial biodiversity interacts with their hosts' physiology is essential for understanding animal ecology and evolution [1]. Microbial communities often influence their hosts' physiology to cope with environmental variation across habitats [2]. Reef-building corals (Cnidaria: Scleractinia) form a symbiotic association with dinoflagellates, which allow corals to thrive on the ocean's euphotic zone along a strong depth-mediated light gradient [3]. Corals living at different depths possess distinctive physiological and morphological traits to optimize energy acquisition which results from genotypic and phenotypic variation within and between coral species [4,5]. Coral colonies at different depths may host distinctive symbiotic algae with contrasting photoacclimation capabilities that grant their hosts the ability to thrive in certain light environments [6,7]. Because of these differences in photoacclimation and the prevalence of specific associations with coral hosts, zonation by light has been regarded as a primary form of niche partitioning in symbiotic corals [8].

While the influence of different species of symbiotic algae on the ecophysiology of reef-building corals has been studied, the effect of other coral-associated microorganisms is less well known, especially across depth-segregated species [9,10]. However, the interest in coral-associated microbes and their roles in maintaining health and preventing diseases has increased substantially [11,12]. From an eco-evolutionary perspective, the evidence suggests that coral-associated bacterial assemblages can be highly variable, although 'footprints' of unique microbial assemblages appear to be mediated by a combination of host species and local environmental conditions [9,13]. These patterns indicate that bacterial communities, like photosynthetic dinoflagellates, could also be spatially structured and segregated along environmental gradients.

Recently diverged coral species that differ in their vertical distribution are ideal systems to study the microbiota–animal relationship as a potential basis for habitat specialization. The *Orbicella* species complex, dominant in Caribbean reefs, was regarded as one species with ecotypic variation, but recent research revealed three species partially segregated by depth [14–16]. *Orbicella annularis* (Ellis and Solander, 1786) forms disjunct columns with senescent edges, being consistently found in shallow waters between 1 m and approximately 20 m. *Orbicella franksi* (Gregory, 1895) forms irregular mounds and plates and is typically found deeper than its two sibling species (up to depths of 60 m). *Orbicella faveolata* (Ellis and Solander, 1786) forms massive mounds and can partially overlap with both *O. annularis* and *O. franksi* habitats [14,17]. The three *Orbicella* species are closely related with incomplete lineage sorting across nuclear and mitochondrial markers [15]. The symbiotic dinoflagellate communities [6,18] as well as the photobiology of this species complex have been extensively studied [7,19], enabling the identification of important differences mediated by environmental gradients. The *Orbicella*-associated bacterial communities have also been examined [11,20]. Therefore, this coral species complex offers an ideal system for the study of how species specialize to live in different habitats through adaptive divergence.

Using a reciprocal transplant experiment between shallow and deep environments in Bocas del Toro (Caribbean Panama), we studied adaptive divergence between the two youngest sister species with the most contrasting vertical distribution within the *Orbicella* species complex, *O. annularis* and *O. franksi*. We surveyed colonies for survivorship and characterized the algal symbiont and microbial communities across habitats. We also evaluated if these recently diverged species have also diverged physiologically along depth-mediated light gradients. Our findings suggest that despite being so young (less than 500 k) [21], these two sister species have diverged in photoacclimation capabilities and microbial symbionts to maximize efficiency in their own light environments.

2. Material and methods

2.1. Reciprocal transplantation

To study the effects of depth and light in *O. annularis* and *O. franksi*, 74 unique genotypes (44 of *O. franksi* and 30 of *O. annularis*) were reciprocally transplanted between shallow and deep environments at Bocas

del Toro, Panama (latitude: 9.327222, longitude: −82.203889). The study site is located on the slope of a relative narrow reef protected on all sides by islands and has been monitored for coral spawning for two decades [16]. This location is ideal to study adaptation across depths because the vertical distribution of these species is compressed toward shallow depths compared with other sites in the Caribbean [22,23], although maintaining the typical vertical zonation pattern (*O. annularis* in shallow water between 2.5 and 6 m with greatest abundance at 3 m, and *O. franksi* in deeper water between 3 and 8 m with greatest abundance at 6 m [16]).

In September 2014, fully pigmented coral clonemate fragments (approx. 5 cm in diameter) were collected from the edges of *O. franksi* colonies ($n = 44$ donor colonies) and vertically oriented colonies of *O. annularis* ($n = 30$ donor colonies), each donor colony being a different genotype (74 unique genotypes in total). Coral fragments were collected from two depths in which each species was abundant: shallow for *O. annularis* (3–4 m) and deep for *O. franksi* (7–8 m). Species identification was performed based on morphological features [14] and represented different genotypes following the multi-locus genotyping work conducted by Levitan *et al*. [16]. Genotypic identity was indicated with unique ID tags attached to each colony. Coral fragments from each species were initially transplanted to polyvinyl chloride (PVC) panels placed near the original depth of collection (3.5 and 9.5 m) where they were left to heal and acclimatize for one week. Subsequently, *O. annularis* colonies were transplanted from shallow to shallow (S-S) ($n = 27$) and shallow to deep (S-D) ($n = 30$). Similarly, *O. franksi* colonies were transplanted from deep to shallow (D-S) ($n = 44$) and deep to deep (D-D) ($n = 28$). The number of fragments transplanted to native environments was lower compared with the ones transplanted to opposite depths because we expected lower mortality in the native environments. We, however, reciprocally transplanted only unique genotypes.

To test for differential mortality across depths, we visually inspected colonies six months after transplantation in March 2015. One detached individual from *O. annularis* transplanted deep was discarded from all subsequent analysis. A one-tailed Fisher exact test was used to assess differences in survivorship among sites. To standardize the fitness (i.e. survival) advantage on the original depth over the opposite depth for each species, differences in fitness were divided over the average fitness on each particular habitat [24].

Samples were collected in accordance with local regulations under CITES permits PWS2014-AU-002155 and 12US784243/9 and Panama permit number SE/A-94-13.

2.2. Environmental parameters

To characterize the effect of the water optical properties on light availability across depths, we measured the diffuse attenuation coefficient for downwelling irradiance (K_d) at the beginning of the experiment. K_d was calculated by measuring changes in light intensities across the depth gradient using the cosine-corrected photosynthetically active radiation (PAR) sensor of a diving-pulse amplitude modulated (PAM) (Walz), previously calibrated against a manufacturer-calibrated quantum sensor (LI-1400, LI-COR). The available light intensity at the depth of each transplant site, expressed as the percentage of incident light, was calculated based on the local K_d as $E_z = E_0\, e^{-Kdz}$ [25], where E_z is the % irradiance at z depth (in metres) and E_0 is the % irradiance at sea surface (100%). Variation in temperature and relative light levels throughout the duration of the experiment was recorded every 30 min from 26 September 2014 until 20 March 2015 by Onset HOBO data loggers (UA-002-64, Onset Computer Corporation) attached to the PVC panels (one logger per panel).

2.3. Photophysiology

To test how depth-dependent light variation affects the photosynthetic condition of corals' symbiotic algae, we measured the chlorophyll *a* (Chl *a*) fluorescence on coral fragments from the transplant experiment and on random colonies across the vertical distribution range of both species, using PAM fluorometry (diving-PAM). Measurements were recorded on 10 fragments (different genotypes) of each species at each depth before transplantation, and every 2–3 days during the week after transplantation. The effective quantum yield ($\Delta F/F_m'$) of photosystem II (PSII) was recorded at noon during peak sunlight exposure and the maximum quantum yield of PSII (F_v/F_m) at dusk. The maximum excitation pressure over PSII (Q_m) was calculated as $Q_m = 1 - [(\Delta F/F_m')/(F_v/F_m)]$ [8]. $\Delta F/F_m'$ was also recorded *in situ* on coral colonies of *O. annularis* ($n = 38$) and *O. franksi* ($n = 67$) randomly distributed over the full depth range of each species. In order to calculate Q_m on these colonies, we estimated F_v/F_m based on a linear regression with data obtained from a subsample of colonies

randomly distributed over the same depth range ($n = 10$ and $n = 21$ for *O. annularis* and *O. franksi*, respectively) (electronic supplementary material, table S1). Pearson's correlation coefficients revealed a strong positive correlation between F_v/F_m and depth in both *O. annularis* and *O. franksi* ($R^2 = 0.85$, $p < 0.01$ and $R^2 = 0.83$, $p < 0.01$), indicating a reliable prediction of F_v/F_m across depths. We used linear regression models to explore the relationship between Q_m and depth for *O. annularis* and *O. franksi* based on evidence that Q_m varies in a pattern that is roughly linear with depth in other coral species [8]. An analysis of covariance was conducted to test for differences in slopes and intercepts among regression models (interaction of species with depth). Due to technical issues with the diving-PAM (loss in hermeticity), samples from the transplant experiment were transported from the transplant sites to the boat in a dark container to record measurements. During this short period of dark acclimatization (less than 5 min), some components of the non-photochemical quenching could have relaxed [26], leading to a slight, yet nearly constant, underestimation of the $\Delta F/F_m'$ recorded at noon and, as a result, of Q_m in all corals. Analyses were conducted using R v. 3.6.1 [27].

2.4. Microbiome

2.4.1. Small subunit ribosomal RNA (16S) amplicon library preparation and sequencing, sequence quality control and initial data processing

We quantified coral-associated microbiome communities in coral transplants to test if adaptive divergence between *O. annularis* and *O. franksi* is in part due to their microbial communities. Tissue samples were collected at the end of the transplant experiment using 1/8″ metal corers by divers wearing nitrile gloves and were immediately deposited in whirl pack bags. Once returned to the boat, each sample was gently washed with filter-sterile (0.2 µm) seawater, deposited in a sterile cryovial and immediately preserved in liquid nitrogen. We extracted DNA from coral tissue samples using the MoBio Powersoil DNA Isolation Kit (MoBio Laboratories). Two-stage amplicon PCR was performed on the V4 region of the 16S small subunit prokaryotic rRNA gene [20,28] (electronic supplementary material). Amplicons were barcoded with Fluidigm barcoded Illumina primers (eight cycles) and pooled in equal concentrations for sequencing. The amplicon pool was purified with AMPure XP beads and sequenced on the Illumina MiSeq sequencing platform at the DNA Services Facility at the University of Illinois at Chicago. Sequences were submitted to the National Center for Biotechnology Information (NCBI) Short Read Archive (SRA) under project number PRJNA717688.

Initial processing of 16S libraries was performed using the Quantitative Insights Into Microbial Ecology (QIIME; v. 1.9) package [29]. Primer sequences were trimmed, paired-end reads merged, and QIIME's default quality-control parameters were used to split libraries among samples. Chimeras were removed and 97%-similarity operational taxonomic units (OTUs) picked using USEARCH 7.0 [30], QIIME's subsampled open-reference OTU-picking protocol [31] and the 97% GreenGenes 13_8 reference database [32]. Taxonomy was assigned using UCLUST and reads were aligned against the GreenGenes database using PyNAST [33]. FastTreeMP [34] was used to create a bacterial phylogeny with constraints defined by the GreenGenes reference phylogeny. OTUs classified as 'unknown' (i.e. sequences not classified at the kingdom level), chloroplast, mitochondria or other potential contaminants were removed. Low coverage samples (less than 223 useable reads) were omitted. Unless otherwise stated, downstream microbiome analyses and figure generation were performed in R v. 3.2.5 [27] using the phyloseq and ggplot2 packages [35,36].

2.4.2. Beta-diversity group significance and differential abundance testing

To quantify differences among treatments, we used weighted UniFrac (wUniFrac) dissimilarity matrices using OTU-level relative abundances. Significant differences in bacterial assemblages were assessed by permutational multivariate analysis of variance (PERMANOVA) with wUniFrac distances and the explanatory variables host species and depth (i.e. vegan::adonis) [37]. Both overall (i.e. *O. annularis* and *O. franksi*) and species-specific models (i.e. *O. annular*is or *O. franksi*) were tested. Heatmaps of OTU abundances were created using the phyloseq::plot_heatmap function [36]. Within-category microbiome variability (i.e. wUniFrac distance) was calculated in QIIME using the make_distance_boxplots function, which also assesses significant differences in microbiome variability among categories via pairwise, non-parametric *t*-tests (1000 Monte Carlo permutations) with Bonferroni correction. To test for significant differences in OTU abundances across host species and depths, we employed negative binomial modelling using DESeq2 [36,38]. Both the overall (i.e. *O. annularis* and *O. franksi*) and species-specific

models (i.e. *O. annular*is or *O. franksi*) were tested. *p*-values for the significance of contrasts were generated based on Wald statistics, and false discovery rates were calculated using the Benjamini–Hochberg procedure.

2.5. Microalgal communities

2.5.1. Internal transcribed spacer 2 rRNA amplicon library preparation, sequencing and initial processing

To quantify differences in dinoflagellate communities across species and depths, we used a two-stage amplicon PCR on the same DNA that was extracted and used for the 16S amplification. We amplified the internal transcribed spacer 2 (ITS2) rRNA marker gene to characterize microalgal taxa within the family Symbiodiniaceae [39]. Once the PCR reactions were finished, samples were held at 4°C before sequencing (electronic supplementary material). Samples were sequenced using the Illumina MiniSeq platform at the DNA Services Facility at the University of Chicago, Illinois. Sequences were submitted to SymPortal for processing and quality checks [40]. Quality checking was performed using mothur [41], followed by taxonomic identification using blastn. The SymPortal pipeline then subdivides sequences into genus groupings and identified type profiles, referred to as defining intragenomic sequence variants (DIVs). Type profiles were only identified if a variant contained more than 200 sequences, and the sequences were subsequently named based on whether they had been used in the definition of the DIVs. The resulting absolute and relative count tables were imported into R v. 3.5.2 [27] for downstream analyses and figure generation using the phyloseq [36], vegan [37], microbiome [42] and ggplot2 [35] packages.

2.5.2. Beta-diversity group significance testing

To compare dinoflagellate communities across samples, we constructed Bray–Curtis and Jaccard dissimilarity matrices using absolute abundances. Significant differences in bacterial communities between sample types were assessed by PERMANOVA with Bray–Curtis and Jaccard distances and explanatory variables including host species, season and depth using the adonis function from the vegan package [37]. We tested overall models that encompassed both species as well as species-specific models.

3. Results

3.1. Temperature and irradiance are higher and more variable in shallow environments

The K_d near the transplant sites was 0.40 m^{-1}, indicating that corals from the shallow (3.5 m) and deep (9.5 m) sites receive, respectively, approximately 25% and approximately 2% sea surface irradiance. Across the vertical distribution range of each species (figure 1*a*), it is estimated that the light intensity varies between 18% and 62% sea surface irradiance for *O. annularis* and between 5% and 33% for *O. franksi*. Relative light levels recorded by data loggers indicated that the light exposure was nearly five times more variable in shallow water than in deep water. Daily temperatures were significantly higher in the shallow site (28.85 ± 0.96°C, mean ± s.d.) than in the deep site (28.46 ± 0.88°C; *t*-value = 3.92, $p < 0.001$; figure 1*b*). However, based on the scaling quotient of temperature (Q_{10}) of *Orbicella* spp. [19], it is estimated that the metabolic rate variation due to differences in temperature among sites is negligible (approx. 5%).

3.2. *Orbicella annularis* experiences greater mortality in deep environments

Transplantation of *O. annularis* S-D ($\Delta_{depth} = 6$ m) resulted in 26% mortality (Fisher exact test: $p = 0.003$) and was significantly higher than that of *O. franksi* colonies transplanted D-D (4% mortality, Fisher exact test: $p = 0.04$). *Orbicella franksi* therefore has an advantage of 26% over *O. annularis* in deep habitats. By contrast, *O. franksi* when transplanted D-S did remarkably well with only 2% mortality (Fisher exact test: $p = 0.63$). Mortality of the two species was not significantly different (0% mortality, Fisher exact test: $p = 0.60$), suggesting that *O. franksi* in shallow areas has no perceivable short-term (less than six months) disadvantage relative to *O. annularis* (Fisher exact test: $p = 0.60$).

3.3. Photoacclimation of *Orbicella annularis* is insufficient to compensate for reduced light

Symbionts of *O. annularis* exhibited a significant increase in F_v/F_m when transplanted S-D (0.622 ± 0.034) relative to corals transplanted S-S (0.541 ± 0.007) (*t*-value = −6.25, $p < 0.01$). On the contrary, symbionts of *O. franksi* transplanted D-S experienced a reduction in F_v/F_m (0.470 ± 0.052) relative to D-D transplants

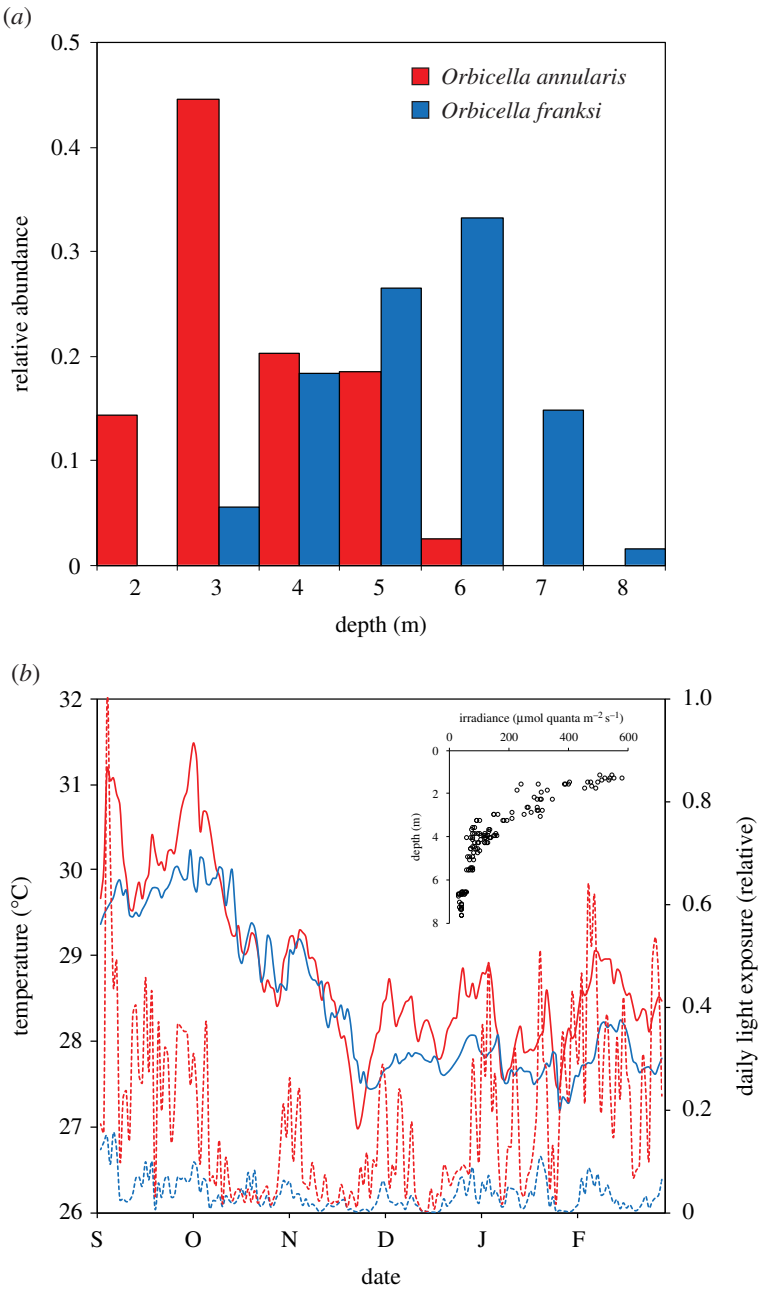

Figure 1. (*a*) Vertical distribution of *O. annularis* and *O. franksi* around the transplant sites in Bocas del Toro, Panamá, previously established as part of the long-term monitoring of coral spawning in which nearly 500 *Orbicella* colonies were tagged and genotyped across the species depth range [16]. (*b*) Variation of the mean daily temperature (continuous lines) and relative light exposure (discontinuous lines) at the shallow (red) and deep (blue) transplant sites. The inset shows the light intensity variation across depths used to calculate the local K_d.

(0.630 ± 0.020; *t*-value = 0.55, $p < 0.01$). Transplantation of *O. annularis* S-D induced a significant reduction in Q_m (0.008 ± 0.076), relative to S-S transplantation (0.216 ± 0.163; *t*-value = 3.67, $p < 0.01$) (figure 2*a*), while *O. franksi* exhibited a significant increase in Q_m (0.226 ± 0.156) when transplanted D-S, relative to D-D transplants (0.073 ± 0.056; *t*-value = 3.26, $p < 0.01$) (figure 2*b*).

Estimations of Q_m on coral colonies along the vertical distribution of each species ranged from 0.099 to 0.673 in *O. annularis* and from 0.020 to 0.492 in *O. franksi* (figure 2*c*). We found a significant species by depth interaction ($F_{1,102} = 28.78$, $p < 0.001$), indicating that the slope of the regression model describing the relationship between Q_m and depth was significantly different between species, being more than twice as pronounced in *O. annularis* ($m = -0.13$; $R^2 = 0.71$, $p < 0.001$) than in *O. franksi* ($m = -0.05$; $R^2 = 0.50$, $p < 0.001$). The linear regression of Q_m with depth indicated that the potential depth limit described by the bioenergetics of the coral–algae symbiosis (i.e. where Q_m reaches the minimum theoretical value of 0) is 5.5 m for *O. annularis* and 7.8 m for *O. franksi* (figure 2*c*), which nearly coincide with the observed lower limit of distribution of both species in the study area (figure 1). Values of F_v/F_m,

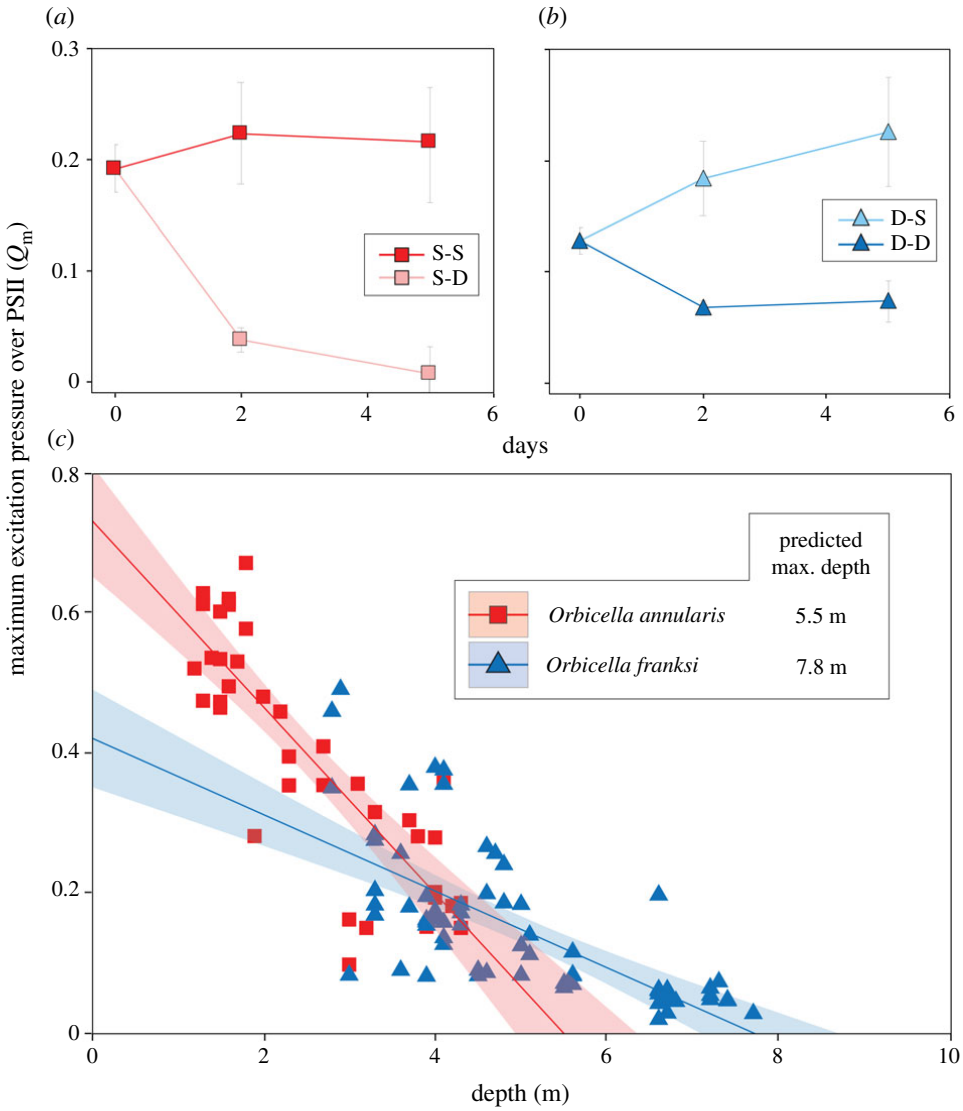

Figure 2. Photoacclimation responses of *Orbicella* spp. across depths. Maximum excitation pressure over PSII (Q_m) is shown pre- and post-transplantation for *O. annularis* (*a*) and *O. franksi* (*b*). Values obtained in *O. annularis* transplanted S-S are shown in dark red while those transplanted S-D in pink. Values from *O. franksi* transplanted D-D are shown in dark blue while those transplanted D-S in light blue. (*c*) Q_m variation in *O. annularis* (red) and *O. franksi* (blue) along a depth gradient. A linear model was used to fit the data and predict the maximum potential depth limit described by Q_m for *O. annularis* ($Q_m = 0.735–0.133 * \text{depth}$; $R^2 = 0.71$, $p < 0.001$) and *O. franksi* ($Q_m = 0.422–0.054 * \text{depth}$; $R^2 = 0.50$, $p < 0.001$). Clear lines represent 95% confidence intervals.

$\Delta F/F_m'$ and Q_m from both transplants and colonies along a depth gradient are provided in the electronic supplementary material, tables S2 and S3.

3.4. Changes in depth produce a major shift in *Orbicella annularis* microbiome

After quality control, sequencing resulted in a total of 577 930 microbial reads (per sample median: 5758; per sample mean: 9173) partitioned across 14 274 unique OTUs. Overall, coral-associated prokaryote communities were significantly structured according to depth ($p = 0.001$), but not host species ($p = 0.12$) or depth by species interaction ($p = 0.86$; PERMANOVA on weighted UniFrac; figure 3; electronic supplementary material, figure S1 and table S4). The change across depths is mainly driven by *O. annularis* ($p = 0.01$, figure 3; electronic supplementary material, table S4 and figure S1). The strong response of *O. annularis* microbiomes to changes in depth can be visualized in differential patterns of OTU abundance among depths (figure 3*a*).

Ten bacterial taxa were significantly enriched in shallow-water samples (electronic supplementary material, table S5). OTUs enriched in shallow-water coral microbiomes are from the bacterial orders Acidimicrobiales (1 OTU), Alteromonadales (1), Kiloniellales (2), Lactobacillales (1), Neisseriales (1), Oceanospirillales (3) and Synechococcales (1). The mean $\log_2$-fold change for enriched OTUs was 5.6.

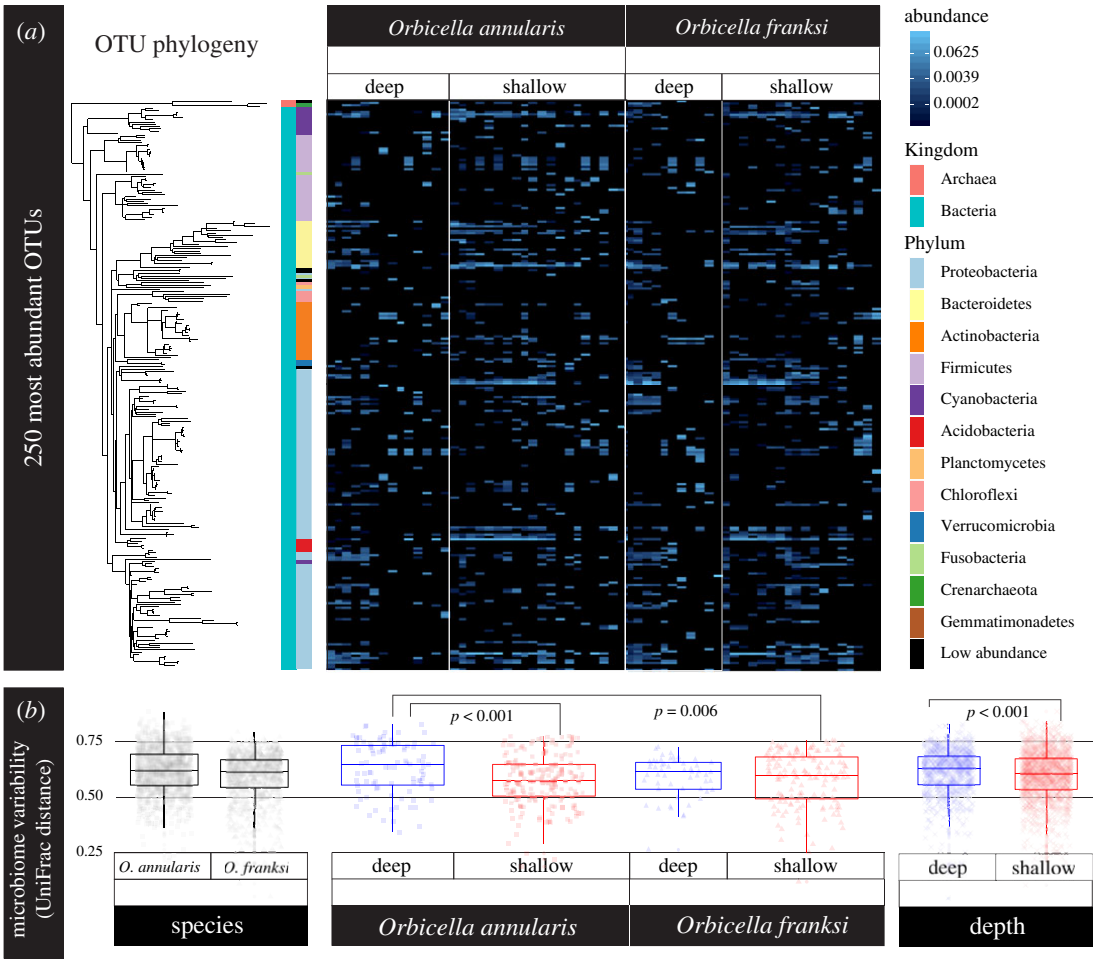

Figure 3. *Orbicella annularis* microbiomes vary across timepoints and depths while *O. franksi* communities remain consistent. (*a*) Relative abundances of the 250 most common OTUs reveal distinct patterns among *O. annularis* microbiomes at the two transplant depths while *O. franksi* abundance patterns remain largely consistent across treatments. Each column in the heatmap represents an individual microbiome sample and phylogenetic relationships among OTUs are shown on the left (FastTree maximum-likelihood tree). (*b*) Microbiome variability (i.e. weighted UniFrac distances) was greatest in *O. annularis* corals transplanted to deep waters. Microbiome variability was higher in corals in deep waters than in shallow.

Microbiome variability did not differ significantly between species with *O. annularis* (0.592 ± 0.008; mean UniFrac distance ± standard error) and *O. franksi* fragments (0.582 ± 0.008) ($p_{adj} = 0.358$). By contrast, microbiome variability differed significantly between depths, being greatest in *O. annularis* transplanted S-D (0.631 ± 0.013; mean UniFrac distance ± s.e.) and significantly higher than *O. annularis* transplanted S-S (0.574 ± 0.008; $p_{adj} < 0.001$) or *O. franksi* transplanted D-S (0.580 ± 0.010; $p_{adj} = 0.006$) (figure 3*b*). The larger microbiome variability in *O. annularis* transplanted deep is consistent with higher mortality and limited photoacclimation potential.

3.5. Symbiodiniaceae communities vary across species

Algal communities of *O. annularis* were significantly different from those of *O. franksi* regardless of the depth to which they were transplanted ($p < 0.05$; pairwise PERMANOVA on a Bray–Curtis matrix, electronic supplementary material, figure S2). Symbiodiniaceae genotypes belonging to the genera *Symbiodinium* (ITS2 type A3) and *Cladocopium* (C3an, C3an/C3, C7 and C7f) occurred in both coral species, although *Cladocopium* genotypes were more abundant in *O. franksi*. Genotypes from the genus *Breviolum* (B1 and B1/B1t) were detected in high abundance in *O. annularis*, and in many colonies from the shallow site (40% of them) were the only dominant symbiont. Only one *O. franksi* colony transplanted D-S hosted a *Breviolum* (B1) population. Genotypes belonging to the genus *Durusdinium* (D1, D1bl, D4 and D4c) were detected only in *O. franksi* transplanted D-D (figure 4). Neither *O. annularis* nor *O. franksi* Symbiodiniaceae communities were significantly different when transplanted to a different depth ($p > 0.1$; pairwise PERMANOVA on a Bray–Curtis matrix).

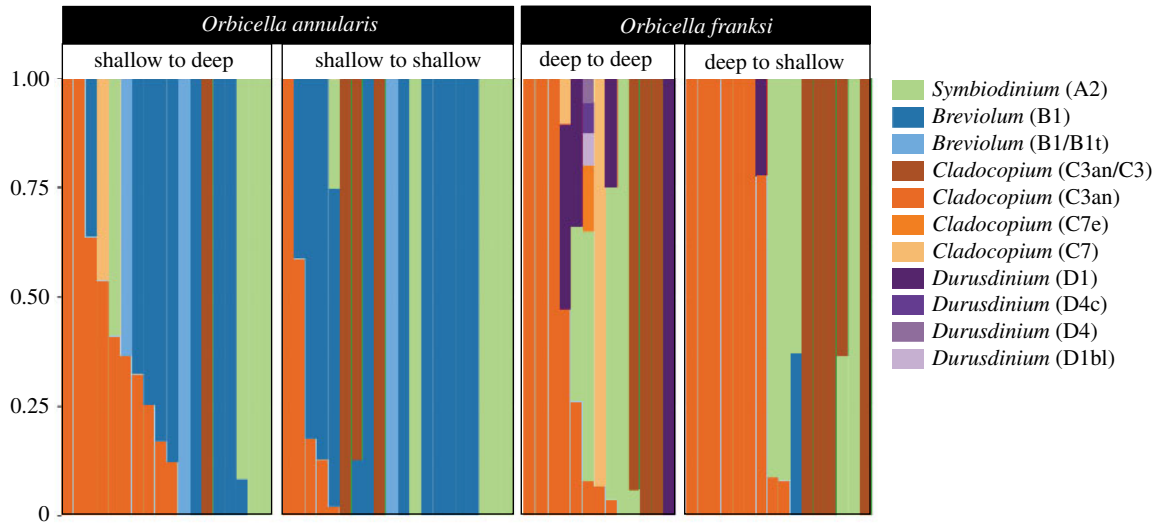

Figure 4. Relative abundance bar plot of Symbiodiniaceae ITS2 profiles identified in *Orbicella* spp. by SymPortal [40]. Variation in Symbiodiniaceae types is shown by species as well as by depth.

4. Discussion

Our study demonstrates that despite being genetically close [16] (electronic supplementary material, figure S3), *O. annularis* and *O. franksi* have diverged physiologically and occupy distinct light environments in part due to the variation in their associated microbiotas (Symbiodiniaceae and bacterial communities). Following transplantation to deep habitats, *O. annularis* experiences a limited photoacclimation potential and disruption of the photosynthetic performance of its algal symbionts, consistent with increased mortality and significant microbiome community shifts with increased variability. By contrast, *O. franksi* maintained a robust physiological performance, a resilient microbiome composition with no significant community shifts or increased variability, and low mortality at both depths. Our study suggests that *O. annularis* is adapted to shallow environments characterized by a higher and more variable temperature and light regimes, while *O. franksi* is physiologically able to live in both shallow and deep habitats. The niches of these sibling species have diverged, and a large component of the niche separation seems to be related to variations in the photoacclimation capabilities and the microbial community of each species. The absence of *O. franksi* in shallow areas may be related to other ecological aspects not considered in our experiment, such as slow growth in an area of intense space competition, a restricted morphological plasticity for regulating the light capture (see below) and/or disadvantages at the larval or recruitment stages in shallow waters.

4.1. The vertical distribution couples with the photoacclimation capabilities of each species

The vertical distribution of *O. annularis* and *O. franksi* is compressed toward shallower depths in Bocas del Toro compared with other clear-water sites in the Caribbean (e.g. Curaçao [22] and Belize [23]). The vertical habitat compression in both species is consistent with the K_d measured in Bocas del Toro ($0.40\ m^{-1}$), which is notably higher than in clear-water sites ($0.06\ m^{-1}$ in Curaçao and $0.08\ m^{-1}$ in Belize [4,43]) and reflects the effect of the heavy rainfall patterns and run-off in the region on the optical properties of the water column [44]. This vertical habitat compression is consistent with other coral reefs exposed to water turbidity [45,46] and suggests that the light penetration into the water column associated with the local K_d is a determinant factor for the vertical zonation of *Orbicella* spp. Despite local differences in the vertical distribution ranges, *O. annularis* consistently occupies well-lit shallow areas of reefs where the potential for increased photosynthesis and calcification rates drives a steep competition for space with other corals. By contrast, *O. franksi* consistently dominates deeper reef areas characterized by low-light conditions and reduced coral growth rates [47].

Our findings indicate that *O. annularis* experiences an almost complete loss of photosynthetic activity when transplanted deep. *Orbicella annularis* fragments photoacclimate to low-light conditions by increasing the light energy conversion efficiency (i.e. increase in F_v/F_m) [48,49]. However, the extremely low values of Q_m reflect a trivial photosynthetic contribution of *O. annularis* symbionts to

the host metabolism due to light-limited photosynthesis [8], suggesting that the photoacclimation potential is insufficient to compensate for the low-light conditions of deep environments. The higher mortality of *O. annularis* in the deep environment supports the insufficient autotrophic contribution to the host metabolism. Photoacclimation of *O. franksi* fragments transplanted to the shallow environment resulted in an increased fraction of photo-inactivated PSII reaction centres and capacity for thermal dissipation of excessive light energy absorbed [48,49]. But in contrast with *O. annularis*, the estimated Q_m in *O. franksi* does not indicate the occurrence of chronic photoinhibition in the shallow environment nor light-limitation in the deep environment, suggesting that *O. franksi* can maintain a more robust physiological performance across depths. The photoacclimation responses of both species in the transplant experiment were consistent with the rates of change in Q_m across their vertical distribution range, which collectively suggest that the symbiotic algae of *O. annularis* are more sensitive to changes in light intensity with depth than symbionts of *O. franksi*.

Colony morphology can help modulate the light capture and photosynthetic energy acquisition along the vertical distribution range of corals [5,50]. The *Orbicella* species complex is unusual among other scleractinians in that they can simultaneously host multi-species communities of symbiotic dinoflagellates, whose composition follows environmental gradients of irradiance within colonies and across depths [6,18]. The dominance of *O. annularis* in shallow habitats correlates with its faster vertical growth among *Orbicella* species and with symbiotic partnerships with particular photoacclimation potential [14]. Its morphology (typically columnar) helps regulate the distribution of light energy for symbiotic algae across the colony surface, representing an advantageous strategy in high-light environments because it reduces the coral tissue area subjected to excessive irradiance [50]. When transplanted deep, this morphology may lead to acute light energy limitation which, in combination with the insufficient acclimatization potential to compensate for low light, can lead to negative energetic balances for the whole colony and eventual death. *Orbicella franksi*, on the other hand, produce plate-like colonies to maximize light capture in deep environments. When transplanted to shallow well-lit environments, despite a potential for successful photoacclimation as indicated by our results, the plate-like morphology limits the capacity to regulate the internal light climate and allows very slow vertical growth. This slow growth makes *O. franksi* a poor competitor, probably explaining why this species is rare in shallow areas. Additionally, disadvantages at the larval or recruitment stage [51] may keep this species from being dominant in shallow waters. In fact, adaptation and strong selection across depths may have promoted the evolution of habitat choice in these *Orbicella* species, as seems to happen in other anthozoans [52].

Another aspect that may influence the vertical distribution of *Orbicella* species is their heterotrophic feeding capacity. It has been indicated that corals from deep environments increase their metabolic reliance on heterotrophy to compensate for reduced photosynthesis [53,54]. The dominance of *O. franksi* in deep environments might be related to an increased heterotrophic feeding capacity relative to its sibling species. Unevenly distributed corallites that give the bumpy appearance to *O. franksi* colonies with larger corallites [14] may confer this species an advantage for passive suspension feeding [55]. However, results from previous studies indicate that colonies of *O. franksi* grow near the lowest growth potential determined by light availability in deep environments [56], suggesting that energy from heterotrophy may not be sufficient to maintain coral growth. The typical plate-like morphology of this species instead of increasing the efficiency of passive suspension feeding is an adaptation to maximize light capture in deep low-light habitats [57]. Overall, the evidence regarding the role of heterotrophy on the vertical distribution of *Orbicella* species is conflicting and inconclusive.

4.2. Host species drive symbiont communities

Species-specific associations with algal symbionts with contrasting photoacclimation capabilities may be a key axis of differentiation between *O. annularis* and *O. franksi*. Despite the higher and more variable temperature and light intensity in shallow areas, *Durusdinium trenchii* was not detected in *O. annularis* colonies. This symbiont is common among *Orbicella* and other coral species growing under harsh environmental conditions [6,58]. Surprisingly, this thermotolerant symbiont (ITS type D1/D1bl) was found in nearly 20% of *O. franksi* colonies from the deep environment. The increased abundance of *D. trenchii* in *O. franksi* may be related to the run-off impacts in the water column (e.g. sedimentation and nutrient enrichment), a reduction in light penetration and the mechanisms by which the coral–algae symbiosis interact with these environmental conditions [59]. The prevalence of *Breviolum* genotypes in *O. annularis* and *Cladocopium* genotypes in *O. franksi*, both in the shallow and deep transplant sites, is consistent with previous reports [59,60] and may indicate the formation of stable associations explained by the photoacclimative capabilities of dinoflagellates and the variability of

physical factors within the vertical distribution range of each coral species [8,60]. The ITS2 analysis has a low resolution to differentiate linages within the same genus in symbiotic algal communities [61,62]. It is possible that complementary analysis with other molecular markers improves the phylogenetic resolution of Symbiodiniaceae (i.e. species or population level), detecting differences in cryptic species/populations of *Cladocopium* spp. or *Breviolum* spp. uniquely associated with each *Orbicella* species like in other depth-segregated anthozoans (e.g. the octocoral *Eunicea flexuosa* and scleractinians of the genus *Leptoseris* spp.) [52,63].

4.3. Microbiome communities vary across depths and are enriched in shallow habitats

Several Endozoicomonas OTUs were significantly enriched in shallow habitats. Endozoicimonaceae are diverse gammaproteobacterial symbionts of numerous marine hosts at varying depths and with a wide global distribution [64]. Members of this group are found in abundance in the tissues of coral species and are considered to be true symbionts of corals which may provide a beneficial function [10,65]. Although their function within the coral host is not entirely clear, proposed benefits include nutrient acquisition, microbiome structuring and roles in coral health.

Members of the family Alteromonadaceae and the order Acidimicrobiales were also enriched in shallow areas. Alteromonadaceae belong to a diverse group of heterotrophic gammaproteobacteria known to associate with marine hosts and nutrient-rich environments. Members of this group tolerate relatively high temperatures and have been used in coral probiotic studies as coral-associated bacteria capable of scavenging free radicals [66] and therefore could provide similar benefits in shallow, high-light environments. Similarly, Acidimicrobiales are known to be planktonic free-living photo-heterotrophs found in both temporal and tropical photic zones [67] and are associated with dissolved organic matter (DOM) in marine environments [68].

Finally, corals in shallow areas were also enriched for *Alloiococcus* and *Synechococcus*. *Alloiococcus* belongs to the group of gram-positive lactic acid bacteria, which are recognized for producing bacterial growth inhibitors that function to deter invading bacteria in their hosts [69]. *Synechococcus* is a photoautotrophic cyanobacterium found in surface waters harbouring abundant light. Both corals and their symbiotic algae are known to actively feed on *Synechococcus* [70,71] which is often found as a member of the coral surface mucus microbiome [13]. As a food for corals, it has been suggested that nitrogen-rich *Synechococcus* cells may increase bleaching recovery and coral health [72].

There is a continuing debate as to the relative role of coral host versus environment in shaping coral microbiomes. This study demonstrates that the responsiveness of coral microbiomes to environmental conditions differs significantly even among very closely related coral species (electronic supplementary material, figure S3). These differences in microbiome shifts may be related to the resilience of the coral host and its associated algal community to a particular habitat. Pantos *et al*. [10] found that environment is the major driver of microbiome structure in *Seriatopora hysterix*, not host genotype or Symbiodiniaceae strain. Our results do not contradict this finding but suggest that responsiveness to environmental conditions can differ significantly even among very closely related coral taxa.

4.4. Implications for coral reef conservation

A key finding in our study with implications for coral restoration is the increased mortality of *O. annularis* when transplanted to low-light environments. We suggest that to enhance survivorship during restoration, the particular light environment of source populations should be similar to the transplant sites. In this study, due to the high vertical attenuation of light ($K_d = 0.40\ m^{-1}$), a 6 m increase in depth resulted in an order of magnitude reduction in irradiance and increased mortality of *O. annularis* by 26%. In a clear-water site (e.g. $K_d = 0.06\ m^{-1}$), this response would be expected to occur with an increase in depth of approximately 40 m. Given the expensive nature of coral restoration, equating the light environment of donor and transplant sites will probably increase yield and decrease costs. Minimally, our approach can be used to estimate the maximum theoretical depth for each species in a given location with certain water optical quality, thereby providing guidance when choosing the location and depth for coral transplantation.

The second aspect of our findings is related to microbiome composition in different habitats across reefs. Our study suggests that the microbiome of shallow-water specialists, like *O. annularis*, may be adapted to an environment with strong changes in light, temperature and salinity. A potential, and relatively unexplored, outcome from ongoing environmental change and disturbances on reef corals, is the instability of associated microbiomes across the vertical distribution range of species. Our study

suggests that the microbiome of shallow-water species respond strongly to environmental change, with potential detrimental effects on the corals' survival. The instability in the coral microbiomes of shallow-water corals will potentially increase as a result of climate change, contributing to the ongoing coral decline in these reef areas [73,74].

Lastly, subtle differences in the water optical conditions can result in changes in the underwater light environment and the vertical distribution of coral species. Most coral reefs around the globe are currently threatened by the direct effects of sediments, pollutants and nutrients associated with coastal development and terrestrial run-off [75]. These conditions affect the water optical quality and, as a consequence, the light climate of corals and the survivorship of species at different depths. Although previous studies have suggested that deep-water species are more sensitive to changes in water optical conditions [4], our results suggest that at least some shallow-water specialists, like *O. annularis*, can be extremely vulnerable to these changes as their physiology/morphology is specialized for high-light habitats. As the degradation of water optical properties in coral reefs continue, shallow-water specialists, which are typically major reef-building species, will probably become rare, shifting the structural and functional integrity of reefs.

5. Conclusion

Our study suggests that the sibling coral species, *O. annularis* and *O. franksi*, are adapted to distinctive light environments along depth gradients. The limited photoacclimation potential and less robust microbiome community restricts *O. annularis* to shallow, high-light environments. *Orbicella franksi* is more versatile, but other ecological aspects such as slow growth in areas of intense space competition restrict the species to deep environments. These contrasting responses associated with the microbial communities highlight the importance of niche specialization in symbiotic corals for the maintenance of species diversity. Our study has implications for coral reef restoration efforts, providing guidance when choosing the location, depth and light environment for coral transplantation.

Data accessibility. Data generated and analysed during the current study, as well as the code used to run these analyses, are openly available in the Figshare digital repository https://doi.org/10.6084/m9.figshare.14687544. Microbiome sequences were submitted to the NCBI SRA under project no. PRJNA717688.

The data are provided in the electronic supplementary material [76].

Authors' contributions. C.P.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, visualization, writing—original draft and writing—review and editing; T.L.-L.: data curation, formal analysis, investigation, methodology, visualization, writing—original draft and writing—review and editing; F.J.P.: data curation, formal analysis, investigation, visualization, writing—original draft and writing—review and editing; S.R.: data curation, formal analysis, investigation, visualization and writing—review and editing; K.B.R.: investigation and writing—review and editing; D.R.L.: investigation, methodology and writing—review and editing; N.K.: investigation, methodology and writing—review and editing; C.W.: funding acquisition, investigation and writing—review and editing; R.I.-P.: funding acquisition, investigation, methodology, resources and writing—review and editing; M.M.: conceptualization, funding acquisition, investigation, project administration, resources, supervision and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

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