

SHORT COMMUNICATION

Highly structured prokaryote communities exist within the skeleton of coral colonies

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Physiological performance, disease and bleaching prevalence are often patchy within individual coral colonies. These responses are largely influenced by coral-associated microbes, but how the coral microbiome changes over small spatial scales has never been quantified before. We performed a high-resolution quantification of the spatial scale of microbial species turnover (β -diversity) within skeletons of boulder-forming *Porites* corals. We found very strong prokaryotic species turnover across spatial scales ranging from 4 mm to 2 m within individual colonies, possibly resulting from dispersal limitation and microbial interactions. The microalgal community was more homogeneously distributed, which is likely due to these photosymbionts actively boring through limestone. Our findings highlight unprecedented levels of intra-colony heterogeneity in the skeletal microbiome, which has implications for the experimental design of coral microbiome studies and for our understanding of coral resilience.

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The vulnerability of corals to pathogens and climate change varies across spatial scales. Thermal tolerance, for example, varies among conspecific corals and within individual colonies depending on their associated *Symbiodinium* community (Rowan *et al.*, 1997; Abrego *et al.*, 2008). Similarly, bleaching susceptibility and diseases such as patchy necrosis are not uniformly distributed within individual colonies (Bythell *et al.*, 2004; Shapiro *et al.*, 2016). Coral health depends on microorganisms, but the existence and consequences of small-scale microbial structure within colonies are virtually unknown. Unveiling fine-scale variation in community composition (β -diversity) within colonies is a critical step towards understanding functional interactions between corals and microbes, and for designing sampling strategies for coral microbiome studies.

The eukaryotic and prokaryotic microorganisms living inside coral skeletons—the endolithic microbiome—have recently come into the spotlight due to their newly discovered biodiversity and potential roles in coral nutrition, nitrogen cycling, bioerosion and coral disease (Schlichter *et al.*, 1995; Tribollet, 2008; Miller *et al.*, 2011; Marcelino and Verbruggen, 2016; Del Campo *et al.*, 2017; Marcelino *et al.*, 2017). Here, we investigated the spatial distribution of prokaryotic and eukaryotic microbiota in the

skeletons of individual boulder corals (genus *Porites*). The decrease in community similarity with distance inside the skeletons was quantified using distance–decay relationships (DDR), a measure of β -diversity and habitat heterogeneity (Nekola and White, 1999; Anderson *et al.*, 2011; Martiny *et al.*, 2011). We collected 90 skeleton samples from healthy, large *Porites lutea* (3) and *P. lobata* (5) colonies following a geometric progression sampling design with distances between samples ranging from 4 mm to 1.992 m (Supplementary Materials, Supplementary Figure S1 and Supplementary Tables S1 and S2). Because our goal was to characterise how community composition changes with distance, we minimised potential environmental differences by collecting each microbiome sample at equivalent positions and the same depth within colonies. Using multi-marker metabarcoding (Marcelino and Verbruggen, 2016) we characterised the prokaryotes (16S rRNA gene marker), eukaryotic microalgae and cyanobacteria (UPA and *tufA* markers—referred to as ‘algae’ hereafter) of each skeleton sample. We quantified within-colony β -diversity as the slope of the linear regression between (\log_{10} -transformed) pairwise community similarities and (\log_{10} -transformed) spatial distances, that is, the slope of the DDR (Supplementary Materials).

Endolithic prokaryotes showed a significant decay in community similarity with distance, suggesting a highly heterogeneous assemblage within individual colonies (Figure 1). The observed DDR slope for prokaryotes (-0.15 , $P = 0.0001$) is among the steepest ever reported for microorganisms (Supplementary

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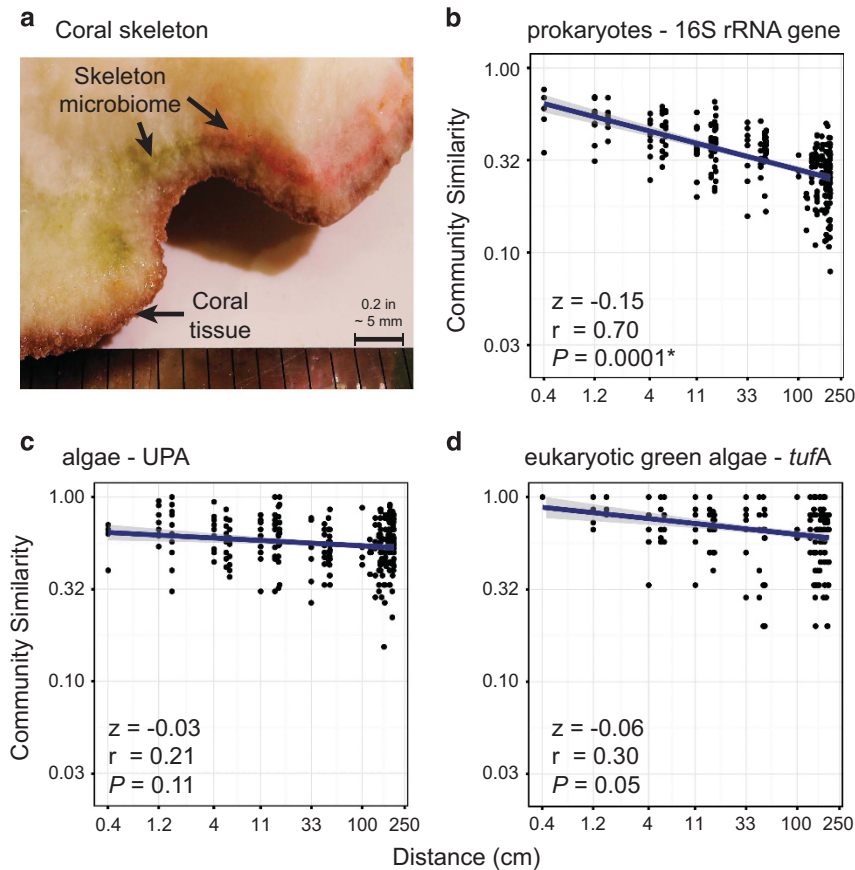


Figure 1 Distance–decay relationships for the endolithic communities in coral skeletons at the intra-colony scale. (a) Coral fragment showing green and red patches of pigmented bacteria and algae. (b–d) Distance–decay relationships obtained with the 16S rRNA gene, UPA and *tufA* markers, respectively. Blue lines indicate the linear regression between (\log_{10} transformed) spatial distance and (\log_{10} transformed) Sørensen community similarity—that is, the slope of the DDR. Shaded areas represent the 95% confidence interval. Slope values (z), Mantel r statistics (r) and significance values (P) are provided.

Tables S3–S5). The DDR slope for bacteria in marine sediments, for example, measured at larger spatial scales, is half of the slope observed here (Zinger *et al.*, 2014; Supplementary Table S5). In contrast, endolithic algae had a shallow DDR slope that was not significantly different from zero, suggesting they were more homogeneously distributed (Figure 1 and Supplementary Table S3). The same pattern holds when individual colonies from different locations are analysed separately, thus it is unlikely that the pattern results from environmental gradients along the colony (Supplementary Figure S2 and Supplementary Table S4). Species accumulation curves confirmed the patchy distribution of endolithic prokaryotes (Supplementary Figures S3–S5). Only a fraction of the colony’s microbial diversity was observed in each $\sim 0.25 \text{ cm}^3$ sample: 26.36% (± 10.77 s.d.) for the 16S rRNA gene, 37.76% (± 15.63 s.d.) for UPA and 51.78% (± 16.67 s.d.) for *tufA*.

The high rate of prokaryotic species turnover may be the consequence of limited dispersal within coral colonies. Microbial motility is likely reduced in dense substrates such as limestone skeletons and aggregation of bacterial colonies may result in high

β -diversity. In contrast, many species of endolithic algae actively bore their way through limestone (Tribollet, 2008) and their higher dispersal ability explains the low algal β -diversity (Figure 1). Microbial interactions and competitive sorting can result in micro-niches with different chemical properties at very small spatial scales and may also contribute to the high prokaryotic β -diversity observed here (see Nekola and White, 1999; Cordero and Datta, 2016).

The high turnover of prokaryotic species raises the question whether different lineages may fulfil similar functions in different parts of the skeleton. While amplicon data cannot answer this definitively, we did observe that a phylogeny-based distance metric produces a shallower DDR slope (Supplementary Materials and Supplementary Table S3). This suggests that while individual species have strong spatial turnover, the phylogenetic lineages to which they belong are widespread. Assuming that basic metabolic functions are conserved among related species, we can expect functional redundancy in the coral skeleton.

The ‘insurance hypothesis’ states that diversity and functional redundancy increase ecosystem stability given species’ functional complementarity and

asynchronous responses to stress (Yachi and Loreau, 1999; Loreau and de Mazancourt, 2013). Extrapolating from this hypothesis, one could expect that spatial structure is associated with patchy responses to stress in the short term, but lineages with similar ecological functions will outcompete their less resilient neighbours conferring ecosystem stability in the long run. It is reasonable to expect therefore that functional redundancy and spatial structure confers an increased long-term stability for the endolithic microbiome. This is a possible mechanism for the apparently stable nature of the coral endolithic community composition, and is in agreement with the hypothesis that the skeleton serves as a stable reservoir for the coral microbiome (Marcelino *et al.*, 2017).

This study provides the first quantitative evidence for turnover of prokaryotic species in coral skeletons at the centimetre scale. We propose that limited dispersal and non-random associations between microorganisms drive the spatial pattern of prokaryotes, while the limestone-boring nature of endolithic algae explains their low species turnover. Whether coral tissue and mucus have similarly high β -diversity still needs to be investigated, but studies indicate that heterogeneity also exist there (Rohwer *et al.*, 2002; Hansson *et al.*, 2009; Daniels *et al.*, 2011). Our findings imply that multiple samples are needed for a full characterisation of a coral skeleton's microbiome and its ecological roles. The notion that microbiome structuring in corals occurs over far smaller spatial scales than previously thought should prompt us to investigate coral health and resilience at the small spatial scales where microbial interactions occur.

Conflict of Interest

The authors declare no conflict of interest.

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