Host traits and evolution shape key coral-bacterial symbioses

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Host traits and evolution shape key coral-bacterial symbioses

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Abstract

The success of tropical scleractinian corals depends on their ability to establish symbioses with microbial partners. Host traits and evolution are known to shape the coral microbiome, but to what extent they affect its composition remains unclear. Here, by using twelve coral species representing the complex and robust clades, we show that functional traits and host evolutionary history explain 14% of the tissue and 13% of the skeletal microbiome composition, providing evidence that these predictors contribute to shaping the holobiont in terms of the presence and abundance of key bacterial species. Additionally, our study shows that the coral tissue and skeleton are dominated by rare bacteria and the skeleton can function as a microbial reservoir. Together, we provide novel insights into the processes driving coral-bacterial symbioses along with an improved understanding of the scleractinian tissue and skeleton microbiome.

Introduction

Ecology and evolution shape trait variation across species and populations, influencing host-microbiome associations (Freckleton and Jetz 2009; Bosch and McFall-Ngai 2011). Some of these relationships affect the fitness of the holobiont (Rosenberg et al. 2009), leading to remarkable evolutionary outcomes that have shaped life on Earth. Corals form a holobiont with unicellular microalgae (Symbiodiniaceae) and a diverse range of microbial communities. The coral holobiont is considered to be an independent level of selection (Zilber-Rosenberg and Rosenberg 2008) but our knowledge of the key mechanisms driving host-symbiont assemblages is fragmentary. It has been observed that host evolutionary processes (Pollock et al. 2018), skeletal architecture (Marcelino et al. 2013; Fordyce et al. 2021) and mode of reproduction (Bernasconi et al. 2019; Damjanovic et al. 2019; Epstein et al. 2019) contribute to microbiome composition alongside other factors like season, environment, host health and developmental stage (O'Brien et al. 2020). Although previous studies have built a firm foundation for our knowledge of coral-bacterial associations, disentangling the individual and combined effects of these factors is paramount to understanding microbial community assembly.

Closely related species can harbour microbiota that resembles each other, a pattern known as phylosymbiosis (Lim and Bordenstein 2020), which has been shown for several terrestrial and marine host-microbe systems, including corals (Bouffaud et al. 2014; Pollock et al. 2018; Griffiths et al. 2019; O'Brien et al. 2020). Phylosymbiosis can be driven by a range of mechanisms, among which microbial filtering moderated by evolving host traits, abiotic and biotic ecological interactions of the host, or co-phylogenetic relationship between the host and microbes (Franzeburg et al. 2013; Douglas and Werren 2016). However, its impact on the coral and the fraction of the microbiome involved remains enigmatic.

Several coral traits can affect the microbiome more directly. Coral skeletal architecture differs among species, affecting the physicochemical properties of the colony (Ricci et al. 2019) and microbial biomass (Fordyce et al. 2021). For instance, light scattering is a function of skeletal density (Marcelino et al. 2013) that results in different spatial gradients of light intensity and spectral composition that affect the microbiome (Magnusson et al. 2007; Ralph et al. 2007; Marcelino et al. 2013; Ricci et al. 2021). Coral reproductive traits can influence the early microbiome, which plays key roles during coral

ontogeny and affects the fitness of the host (Damjanovic et al. 2019) and while broadcast spawning corals depend mostly on horizontal transmission (Lesser et al. 2013; Nitschke et al. 2016; Epstein et al. 2019), brooding species exhibit some degree of vertical transmission (Quigley et al. 2018). Alongside reproductive mode and skeletal architecture, a range of host ecological and morphological traits are likely to take part in the establishment and development of the coral microbiome. For instance, interactions between planulae and crustose coralline algae were identified as potential cues influencing coral settlement (Jorissen et al. 2021) and the substrate of attachment is known to influence the holobiont (Massé et al. 2018). But whether coral bacterial communities can be predicted based on host traits is still to be investigated.

The coral microbiome is a dynamic system and an excellent case study of a highly diverse biological community contributing to holobiont success. Unravelling the composition of coral microbiomes is a necessary step towards understanding the interspecies relationships and functioning of the holobiont. The goal of our study was to disentangle the influence of drivers affecting the microbiome composition, with a particular focus on reproductive mode and coral skeletal traits as well as host evolutionary biology. Our experiment was also designed to reduce the variability introduced by a range of factors known to affect the coral holobiont, such as colony age (Williams et al. 2015), spatial-temporal variability (Dunphy et al. 2019) and health status (Maher et al. 2019).

Results

Experimental design

We sampled 12 coral species from the complex and robust clades aiming to disentangle differences in microbiome composition between host anatomical compartments and surrounding environment and to quantify to which extent host phylogeny, skeletal architecture and reproductive mode are associated with microbiome composition. Our sampling design also aimed to reduce factors known to affect the coral holobiont. We sampled only adult healthy colonies from a small geographic area, within three weeks and we characterized their tissue and skeletal prokaryotic communities using 16S rRNA amplicon sequencing. In order to disentangle the influence of host phylogeny, skeletal architecture and

77 reproductive mode on the bacteria residing in the tissue and skeleton (Fig. 1), we performed variation
78 partitioning and canonical correspondence analysis (CCA). Then, we investigated and compared the
79 microbial communities of the coral tissue and skeleton (Fig. 2), as well as surrounding seawater and
80 sediment and assessed differences among groups using multivariate analyses and ordination.

81 Host traits and evolution shape the bacterial community composition

82 We quantified the influence of some of the most significant processes known to take part in the
83 establishment and development of the coral microbiome, like host phylogeny (Pollock et al. 2018),
84 skeletal architecture (Fordyce et al. 2021) and reproductive mode (Damjanovic et al. 2019; Epstein et
85 al. 2019), by applying variation partitioning analysis to the tissue and skeletal bacterial communities.
86 Our models explained 14% of the tissue and 13% of the skeletal microbiome variation, leaving a high
87 proportion of the variation (>85%) unexplained by the predictors (Fig. 3). This is not fully surprising
88 since past reports have found that the coral microbiome is variable within a single coral colony
89 (Damjanovic et al. 2020), among individuals, species and reef habitat (Ainsworth et al. 2015; Hester et
90 al. 2016; Hernandez-Agreda et al. 2016). We argue that a combination of unmeasured environmental
91 variables, functional redundancy, community assembly processes and physicochemical properties
92 specific to each coral colony likely take part in determining the coral holobiont assemblage and limiting
93 its broader consistency.

94 *Host evolution drives key coral bacterial associations*

95 Employing LeFSe analysis, we identified members of the coral microbiome that preferentially
96 colonized the coral tissue or skeleton (Supp. Data 1; Supp. Fig. 1), then through CCA analysis we
97 assessed their association with host phylogenetic history and coral traits. In the CCA analysis the three
98 coral phylogenetic variables (PV1, PV2 and PV3 - see Host phylogeny in M&M) showed a strong
99 association with some ASVs (Fig. 4a and 4b).

100 *Endozoicomonas* preferentially colonized the coral tissue (Supp. Fig. 1) and individual ASVs
101 belonging to this genus were associated with corals in the robust clade (Fig. 4a; positive correlation
102 with phylogenetic variable PV1) and the families Poritidae (Fig. 4a) positive correlation with

phylogenetic variable PV2) and Pocilloporidae (Fig. 4a; positive correlation with phylogenetic variable PV3). Our results confirmed *Endozoicomonas* being a highly prevalent coral symbiont across coral species (Neave et al. 2017; Tandon et al. 2020) and are also in agreement with Pollock et al. (2018), who proposed phylosymbiosis between this bacterial group and corals. Although the knowledge about the processes driving phylosymbiosis is still fragmentary, evidence shows that host-filtering may play a role (Mazel et a. 2018). By assessing patterns of association of entire microbiomes with their hosts, phylosymbiosis also takes into account some long-term host-microbe relationships that have developed into symbioses (O'Brien et al. 2020) and, because of *Endozoicomonas* putative beneficial roles like nutrients provision and aiding host homeostasis (Neave et al. 2016; Tandon et al. 2020), it seems likely that these bacteria developed a mutualistic symbiosis with corals over time.

Alteromonas and *Pseudoalteromonas* preferentially colonized the tissue of robust corals and in the family Pocilloporidae (Fig. 4a; Supp. Fig. 1). Additionally, *Pseudoalteromonas* was associated with the family Poritidae (Fig. 4a). This is the first evidence of phylosymbiosis between these coral lineages and bacterial genera thought to take part in nitrogen cycling and antibacterial activity in the coral holobiont (Shnit-Orland et al. 2012; Ceh et al. 2013a).

Bacteria in the genera *Spirochaeta* and "*Candidatus* Amoebophilus" preferentially colonized the coral skeleton (Supp. Fig. 1) and individual ASVs belonging to these genera were associated with corals in the robust clade and the family Poritidae (Fig. 4b), in line with the results of Pollock et al. (2018). Our data also indicate phylosymbiosis for *Spirochaeta*, bacteria that usually thrive in oxygen deprived environments (Leschine et al. 2006) like the coral skeleton and could be pivotal members of the coral holobiont because of their ability to fix nitrogen and carbon (Lawler et al. 2016). Despite "*Candidatus* Amoebophilus" having been flagged as a member of the coral core microbiome (Ainsworth et al. 2015; Ricci et al. 2021), its role in the holobiont is still unclear. Available data on "*Candidatus* Amoebophilus" (Kantor et al. 2015) and "*Candidatus* Amoebophilus asiaticus" (Schmitz-Esser et al. 2010) show reduced genomes with limited metabolic capabilities, suggesting they may rely on the host for survival. Interestingly, "*Candidatus* Amoebophilus asiaticus" genome harbours a high count of host cell interaction genes, particularly a wide arsenal of eukaryotic domain-like proteins.

These proteins are used by intracellular pathogens to interact with hosts and modulate host response via multitude protein-protein interactions (Schmitz-Esser et al. 2010).

Bacteria in the Myxococcales order preferentially colonized the coral skeleton (Supp. Fig. 1) and were associated with corals in the robust clade (Fig. 4b). Moreover, recent studies have shown that these microbes might have co-diversified with corals (Pollock et al. 2018). Myxococcales are known to play beneficial roles in other systems like agricultural settings (Wang et al. 2020a), where they keep pathogen populations under control by releasing large quantities of antibiotics. The skeleton of many corals analyzed in this study concurrently harboured high abundance of Myxococcales and potential pathogenic bacteria (*i.e. Vibrio* and *Serratia*). Thus, as reported for agricultural settings and given that all the sampled colonies were visibly healthy, the Myxococcales may play similar roles in the coral holobiont by controlling pathogen populations (Rosales et al. 2019).

Skeletal architecture affects the microbial abundance

Skeletal architecture influenced the composition of tissue and skeletal microbiomes (Fig. 3). The skeleton affects the physicochemical properties of the whole coral colony including the tissue and Marcelino et al. (2013) found that, by refracting light back, the skeleton alters the light environment in the tissue, affecting the Symbiodinaceae. One could argue that this also causes downstream effects on other members of the tissue microbiome. Accordingly, we found that skeletal architecture was associated with several key members of the tissue microbiome, including *Endozoicomonas*, *Alteromonas* and *Pseudoalteromonas* (Fig. 4a). Our results also indicate that skeletal architecture alters the abundance of bacterial species rather than filtering them out entirely. For instance, we found that despite the bacterial genera *Bacillus*, *Halomonas*, *Pseudoalteromonas* and *Vibrio* being mainly associated with corals with porous skeletons like *A. aspera* and *M. digitata*, they were also present in species with denser skeletons at low abundance. Similarly, the bacterial families Kiloniellaceae and Hyphomonadaceae, as well as the genus *Rugeria* that we found at higher abundance in species with denser skeletons (e.g. *P. sinensis*, *G. retiformis*), were also present in corals with more porous skeletons at low abundance.

Reproductive mode promotes beneficial functional associations

Our data show that tissue and skeletal microbiomes of scleractinian corals were influenced by the reproductive mode (Fig. 3) and the reproductive mode variables (broadcast spawners, brooders and mixed mode) were associated with key holobiont members, including *Endozoicomonas*, *Alteromonas*, *Pseudoalteromonas* and Myxococcales (Fig. 4a and 4b). These bacteria are all known for their beneficial roles (Shnit-Orland et al. 2012; Ceh et al. 2013a; Neave et al. 2016; Rosales et al. 2019) and possibly benefiting early developmental stages of the coral through nutrient cycling and facilitating early immune system development. But, in the tissue, reproductive mode was also associated with controversial bacteria like *Serratia* and *Vibrio* (Fig. 4a), which are known as potential pathogens in some coral species (Ben-Haim et al. 2002; Alagely et al. 2011; Bernasconi et al. 2019). We argue that these putative pathogens, in normal conditions, are commensal members of the holobiont, while as suggested in previous studies their detrimental potential could emerge during dysbiotic states (Kemp et al. 2018).

Variation partitioning analysis showed that reproductive mode explained a portion of the microbiome variation (tissue: 3% and skeleton: 4%; Fig. 3). Studies investigating the establishment of microbiomes reported that corals can vertically transmit a consortium of bacteria (Leite et al. 2017; Zhou et al. 2017; Damjanovic et al. 2019; Bernasconi et al. 2019; Epstein et al. 2019). Similarly, our CCA analysis showed that some bacterial groups highlighted by these studies (*i.e. Acinetobacter* spp., *Bacillus* spp., Caulobacterales, Cryomorphaceae, Endozoicomonadaceae, *Pseudomonas* spp., Rhizobiales, Rhodobacterales) were likely vertically transmitted in the species analyzed in our study. The correspondence between our findings and past reports shows that coral reproductive mode influences the microbiome composition predictably and that early host-symbiont associations persist across a coral's lifetime.

Besides the microbial taxa reported to be vertically inherited in corals before, our results show that reproductive mode correlated with a range of other bacteria, suggesting they may also get transferred from parents to offspring and, based on their roles in other systems, they can be hypothesized to play roles in host development as well. Among the genera we found to be associated with the

183 reproductive modes mixed and brooders, *Stenotrophomonas* are known to cycle sulfur and nitrogen
184 compounds in plants (Ryan et al. 2009) and *Massilia* are known to stimulate plant growth by producing
185 compounds like siderophores (Ofek et al. 2012). Nutrient cycling and pathogen control seem to be two
186 common functional features among vertically transmitted bacteria, increasing survival rate and fitness
187 of developing corals (Ceh et al. 2013b; Zhou et al. 2017). Thus, by influencing the early microbiome,
188 the reproductive mode could drive host-bacteria functional associations pivotal during coral ontogeny.

189 Coral microbiomes are dominated by rare species

190 Except for a few bacteria that were consistently present across several coral species, rare species
191 dominated the coral microbiomes. The great majority of bacterial ASVs (99.6% for tissue and 99.7%
192 for skeleton) were not consistently present in at least 30% of the samples. The core microbiome, i.e. the
193 ASVs occurring in at least 30% of samples, consisted of only 14 ASVs in the tissue and 9 in the skeleton
194 (Supp. Data 2).

195 A closer look at microbiome variability within species showed that even within closely related
196 hosts most bacterial ASVs were rare, leading to wide variability between samples. The skeletal microbiome
197 showed a higher proportion of core members than the tissue microbiome for 8 out of 12 coral species
198 (Supp. Data 3). Since the microbial communities of tissue and skeleton were dominated by rare bacterial
199 ASVs, we analyzed their Pielou's evenness, a measure indicating how similar the abundances of
200 different species in the microbiome are (Jost 2010). Despite finding comparable evenness of the tissue
201 and skeletal bacterial communities (Supp. Fig. 2a), when we compared across coral species we found
202 high variability in the evenness of both tissue and skeletal microbiome (Supp. Fig. 2b and 2c). For
203 instance, in *G. retiformis* both tissue and skeletal microbiomes showed evenly distributed bacterial
204 populations, while in *P. australensis* the tissue microbiome was dominated by the genus
205 *Endozoicomonas* and the families Caldicoprobacteraceae, Thermoanaerobaculaceae and
206 Cyclobacteriaceae dominated the skeletal microbiome of individual samples. In line with our results,
207 it has been previously reported that the microbiome composition of each coral species can show various
208 degrees of diversity (McDevitt-Irwin et al. 2017) and in some cases one or a few bacterial taxa can be
209 dominant, like in *P. verrucosa*, whose bacterial community is dominated by the genus *Endozoicomonas*

(Voolstra and Ziegler 2020). A range of processes including host evolution (Pollock et al. 2018), traits (Damjanovic et al. 2019; Epstein et al. 2019), microniches partitioning (Ricci et al. 2019), priority effects and functional redundancy (Louca et al. 2016) may synergistically affect the microbiome assembly and ultimately determine the variability of corals' bacterial communities.

Rare bacteria are understudied in many environments, but it is known that their presence and abundance are often driven by environmental variables (Campbell et al. 2011) and this is probably true for the coral holobiont too. Voolstra and Ziegler (2020) suggested that functional redundancy may promote high microbiome flexibility and we argue that this process may also structure the rare portion of the coral microbiome. Although our study design is not geared towards investigating whether the coral microbiome is driven by functional redundancy, our analysis of rare bacteria (observed in <50 reads per sample) that are likely involved in nitrogen and sulfur metabolism suggest that coral samples often featured unique ASVs of these guilds (Supp. Data 4), e.g. nitrogen fixers in the Rizhobiales (Wang et al. 2020b) or sulfate reducers in the Deltaproteobacteria and Thermodesulfovibrionia (Henry et al. 1994; Chen et al. 2021). While these data alone do not prove that functional redundancy is at the basis of the observation that a large proportion of bacterial ASVs in the coral microbiome is very rare, they do suggest that at least in some cases rare bacteria fill functional niches in the coral holobiont.

Coral tissue and skeletal microbiomes overlap but differ quantitatively

Our results are in clear agreement with the hypothesis formulated by Marcelino et al. (2017) that the skeleton can serve as a reservoir for coral tissue microbes. Indeed, our work shows that a large fraction of bacteria can colonize both anatomical compartments (*i.e.* 86% of tissue ASVs were found in at least one skeleton sample and 56% of skeletal ASVs were found in at least one tissue sample). On the basis of this, it can be argued that after a period of dysbiosis, beneficial bacteria could quickly repopulate the tissue from the skeleton (Marcelino et al. 2017). Despite this, the β-diversity of coral tissue and skeletal microbiomes differed significantly for all coral species (p values in range 0.006 - 0.019) except *P. daedalea* (Supp. Fig. 3). Therefore, our results indicate that while many bacteria are not selective in colonizing the coral tissue or skeleton, the complex array of biotic and abiotic

interactions characteristic of each compartment shapes its microbiome composition differently (Fig. 5; Supp. Fig. 3).

Coral tissue and skeleton are also known to differ in their physicochemical environment, with the skeleton offering a wide array of microniches (Ricci et al. 2019) and one study has reported that the skeletal communities are more diverse than their tissue counterparts (Pollock et al. 2018). Our data showed comparable α-diversities of tissue and skeleton microbiomes for most coral species (Supp. Fig. 4). This conflicts with the findings of Pollock et al. (2018) and while it may represent an underlying biological cause, methodological differences between the studies may also contribute to the observed differences (e.g. using ASVs vs. OTUs for taxonomic resolution).

Sourcing the microbiome: vertical and (or) horizontal acquisition

Our results suggest that both the tissue and skeleton have a component of vertically acquired bacteria, whereas it is likely that the remaining microbiome portion is more dynamic and taken up from the environment during different seasons and life stages of the host. Our comparison of the coral microbiota with those of the surrounding seawater and sediment showed that bacterial species shared between corals and their environment at the time of sampling accounted for more than 30% of the ASVs in *A. aspera* and *P. damicornis* (Supp. Data 5). In line with our results, recent studies show that the vertically and horizontally acquired microbiome varies among coral species (Leite et al. 2017; Zhou et al. 2017; Bernasconi et al. 2019; Epstein et al. 2019). For instance, in *P. damicornis* the bacterial community establishment is largely driven by the microbiome present in the environment (Epstein et al. 2019) while in *A. digitifera*, *A. gemmifera* and *Mussismilia hispida*, there is evidence of vertical transmission of a wider microbiome portion (Leite et al. 2017; Zhou et al. 2017; Bernasconi et al. 2019). Moreover, although for some coral species we found a low similarity between their microbiome and that of concurrently sampled seawater and sediment (Supp. Data 5), this by no means implies that environmental acquisition is not an influential process in the establishment of these coral species' microbiome. Given that our seawater and sediment samples were taken over a period of 1 month, it

seems likely that a more prolonged sampling would recover a higher fraction of the coral microbiome in these and other potential environmental reservoirs.

Unusual suspects show persistent associations with corals

Our work identified several bacteria that were consistently associated with corals but are either new to the coral microbiome field or understudied. Cyclobacteriaceae were present in at least one sample of eight coral species except for *A. aspera*, *G. tenuidens*, *I. palifera* and *S. pistillata*. Currently, Cyclobacteriaceae have only been reported in 7-months-old *Acropora* recruits (Chan et al. 2018) and their intra-colony changes in abundance after *Acropora* spp. bleaching (Durante et al. 2019). Our work shows that this family is much more widespread across the complex and robust clades. From a functional point of view, members of this family could benefit the coral through their metabolic capabilities including carbohydrate metabolism, carotenoid biosynthesis, antibiotic resistance and quorum-sensing regulation (Pinnaka and Tanuku 2014).

Bacteria in the genus *Paramaledivibacter* were found in six *P. lutea*, three *P. daedalea* and two *P. australensis* samples. To our knowledge, this is the first time for this genus of strictly anaerobic bacteria to be reported in the coral literature. While at this stage we can only guess as to its functions in the coral holobiont, it might be a harmful species due to its ability to degrade amino acids and peptides (Li et al. 2016). As mentioned above, detrimental effects by pathogens could be counterbalanced by beneficial holobiont members including Myxococcales and *Pseudoalteromonas*. Despite *Paramaledivibacter* being described as strictly anaerobic, we did find these bacteria in the coral tissue. This finding could sound surprising, but this is not the first study reporting obligate anaerobes in coral compartments known to show oxygen presence (Marcelino and Verbruggen 2016; Cai et al. 2017).

Roseospira has also not been reported in the coral literature before, but we found these bacteria in the tissue and skeleton of three *P. lutea,* two *G. retiformis*, one *G. tenuidens* and one *P. daedalea* samples. These purple non-sulfur bacteria seem to be able to colonize a diverse range of environments and optimally grow photoheterotrophically (Guyoneaud et al. 2002). Thus, given their ability of utilizing substrates known to be present in corals like acetate (Patton et al. 1977) and glutamate (Su et

al. 2018) and using near-infrared wavelengths not absorbed by Symbiodiniaceae, the coral colony could offer an array of microniches where these bacteria's niche preferences are met.

Discussion

Through a combination of a homogeneous experimental design that minimizes external biases affecting the microbiome, use of innovative technologies like micro-CT scanning to quantify host traits and the application of a range of statistical analyses, our study allowed us to unravel the structure of the coral microbiome and quantify how it is influenced by host traits and evolution.

We showed a significant association of host evolution and traits with the bacterial community, including a range of host-bacterial relationships known to affect holobiont health and functioning. Based on our results, we hypothesize that reproductive mode could aid vertical transmission of bacteria beneficial to corals' development, while skeletal architecture works like a filter affecting bacteria abundance. Although our analysis accounted for some of the most influential processes known to affect the microbiome composition, these could only marginally explain the microbiome variation of tissue and skeleton. A holistic view of the mechanisms determining the holobiont composition will be gained by incorporating the physicochemical and dynamic biochemical environment of the coral colony and its influence on the structure of the microbiome and also by assessing whether the presence of some bacteria (whether dominant or rare) may influence the overall structure of the microbiome. In this study, we provided substantial evidence that coral tissue and skeletal microbiomes are dominated by rare taxa and differ in compositions, but a consortium of bacteria can colonize both compartments and the skeleton could be a microbial reservoir.

While our study answers several unsolved questions about the bacterial community structure of scleractinian corals and the mechanisms driving its composition, it also exposes knowledge gaps. Despite our study's focus on commonly studied coral species, we identified several abundant bacterial groups that were not previously reported in coral literature. This highlights that the field of coral reef microbial ecology still presents substantial hiatuses even at the level of characterizing the taxonomic composition of the microbiome and substantial further work will be needed to fully characterize the

313 microbiome, understand its functions in the coral holobiont, its fine-scale distribution in relation to
314 ecological micro-niches and the metabolic hand-offs that happen among microbiome members and with
315 the host. The use of putative beneficial microorganisms has been proposed as a tool to mitigate the
316 increasing pressure of anthropogenic activities on coral reefs (Peixoto et al. 2020), therefore we hope
317 that the detailed knowledge about community structure gained in our study can form the basis for further
318 advances in probiotic strategies to improve coral resilience in future climate scenarios.

319 **Material and Methods**

320 Sample collection, processing and statistics

321 All the samples were collected under the permit G19/41658.1 issued by the Great Barrier Reef Marine
322 Park Authority. Seventy-two coral fragments (collected at low tide <1 water depth), five seawater
323 samples (5 L each) and five 50 mL sediment samples were collected from the research zone of Heron
324 Island reef flat, central Great Barrier Reef (GBR; 23°44′S, 151°91′E), during January 2020. Corals were
325 collected using a sterile hammer and chisel and placed in sterile zip-lock polyethylene bags in seawater.
326 Coral tissue was removed from the skeleton of each sample using a waterpik and sterile seawater (SSW),
327 then tissue slurry and skeletal fragments were collected and snap-frozen by immersion in liquid nitrogen
328 and stored at -80 °C until processing. The seawater samples and 2 samples of SSW (5 L each) were
329 filtered using 0.22 µm filters (MilliporeSigma) before snap freezing. By sequencing the tissue and
330 skeletal microbiome of 72 corals (144 samples in total) belonging to 12 species in the complex and
331 robust clades, five seawater and sediment samples each, we retrieved 7,025,745 16S rRNA sequences
332 (sample minimum: 2,073; mean: 46,528; maximum: 115,692). Because of the comparative nature of
333 this study, we took a range of precautions to avoid any potential cross contamination and
334 computationally removed potential contaminants. For instance, we sequenced SSW used to remove the
335 coral tissue contaminants; sampled skeletal fragments 5 mm from the tissue to prevent tissue-skeleton
336 microbiomes cross contaminations; and sequenced control samples taken during the DNA extraction
337 and amplification.

Library preparation, sequencing and initial quality control

The total DNA of each coral tissue, coral skeleton, seawater, sediment and control sample was extracted using the Wizard Genomic DNA Purification Kit (Promega). Extractions were also performed on eight blanks taken during both the extraction and amplification protocols. SSW and blanks served as controls. We used a 2-step PCR amplification, the first amplifying the target marker and the second adding Illumina adapters (underlined). The V5-V6 regions of the 16S rRNA were PCR amplified using the primer pairs: 784F [5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGATTAGATACCCTGGTA -3′] and 1061R [5′- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCRRCACGAGCTGACGAC -3′] (Andersson et al. 2008).

Sequences were processed using the QIIME2 pipeline version 2020.11 (Bolyen et al. 2018). Cutadapt was used to remove primers (Martin 2011). DADA2 was used to merge forward and reverse reads, remove poor-quality sequences, perform dereplication and eliminate chimeras (Callahan et al. 2016). Taxonomy was assigned using the feature-classifier plugin in-built in QIIME2 - SILVA v128 QIIME release (Quast et al. 2012).

Host phylogeny

We confirmed the identity of the targeted corals based on skeletal morphology. The phylogeny of the 12 coral species was extracted from the multigene molecular phylogeny of corals published by Huang and Roy (2015). We took their set of supertrees and derived a consensus tree in TreeAnnotator (BEAST 2.4.8; Bouckaert et al. 2019). We computed a matrix of pairwise patristic distances from the phylogenetic tree with the distTips function from adePhylo (Jombart and Dray 2010) and performed non-metric multidimensional scaling (NMDS, isoMDS from MASS; Venables and Ripley 2002) to obtain a set of variables that can be used to relate the host phylogeny to the microbiome in multivariate analyses. The vectors of the positions of the coral species along the three dimensions of the NMDS were used to represent host phylogeny in downstream analyses dividing it into three main phylogenetic variables. Phylogenetic variable PV1 separated the robust from the complex corals, the robust clade positively correlating with PV1 (*Goniastrea retiformis, Paragoniastrea australensis, Platygyra*

365 *daedalea, Platygyra sinensis, Pocillopora damicornis and Stylophora pistillata*) and negatively with
366 the complex clade (*Acropora aspera, Goniopora tenuidens, Isopora palifera, Montipora digitata,*
367 *Porites annae and Porites lutea*). Phylogenetic variable PV2 represents the phylogenetic subdivision
368 of complex species into Poritidae (*G. tenuidens, P. lutea and P. annae*), which correlated positively
369 with PV2 and the Acroporidae (*A. aspera, I. palifera and M. digitata*), which correlated negatively with
370 PV2. Phylogenetic variable PV3 represents the phylogenetic subdivision of the robust species into
371 Pocilloporidae (*P. damicornis and S. pistillata*) that correlated positively with PV3 and the Faviidae
372 (*G. retiformis, P. australensis, P. daedalea and P. sinensis*) that correlated negatively with PV3.

373 Skeletal architecture

374 Portions of all 72 coral fragments were scanned using a Phoenix Nanotom M micro-CT scanner
375 (Waygate Technologies) capturing the full specimen structure at a voxel resolution of 20 µm. Further
376 to this, a 12 mm x12 mm x12 mm region-of-interest (ROI) from one sample of each of the 12 species
377 were scanned at a 10 µm resolution. Preserved samples were scanned in air and secured within a
378 specimen jar with bubble wrap to prevent movement during scanning. Scans were collected using the
379 datos|x acquisition software (Waygate Technologies) and X-ray energy of 110kV and 300 mA with a
380 tungsten target and 0.1 mm copper filter to pre-harden the X-ray beam. A fast scan setting was used
381 collecting between 1199 to 1798 projections through a full 360° rotation of the specimens, depending
382 upon sample width on the instrument detector, with an integration time of 0.5 seconds per projection
383 leading to a 10 to 15 minute scan time. Large specimens were scanned twice to capture the full specimen
384 structure using a multiscan feature.

385 Micro-CT data was reconstructed using the datos|x reconstruction software (Waygate
386 Technologies) applying an ROI and inline median filter during the reconstruction of the data.
387 Reconstructed data was imported into Avizo version 2019.3 (Thermo Scientific) for analysis. The
388 structure of each coral specimen was evaluated by segmenting three different phases observed in scans,
389 the dense skeletal phase (bright white structure in Supp. Fig. 5a), a lower density organic phase
390 (intermediate gray values in Supp. Fig. 5a) and trapped air within the structure phase (dark gray-black
391 space in Supp. Fig. 5a). The Auto Threshold algorithm of Avizo was used for segmentation of the 3

phases (Supp. Fig. 5b), which is based on a factorization method developed by Otsu (1979) and determines the point for segmentation between phases in the grayscale histogram (Supp. Fig. 5c). To determine the total porosity ($V_{porosity}$, Eq. 1a) of specimens a sample mask was created by using the Closing and Fill Holes operations of Avizo on the segmented skeleton ($V_{skeleton}$, Eq. 1a) plus organic matter ($V_{organic}$, Eq. 1a) to produce a solid sample mask (V_{sample}, Eq. 1a) encompassing the boundaries of the sample. The total volume of segmented air (V_{pores}, Eq. 1b) and organic phase ($V_{organic}$, Eq. 1b) within the sample mask is then taken as a measure of sample porosity (Eq. 1b). Segmented label volumes were calculated using the Volume Fraction algorithm in Avizo, which also determines the volume fraction of each phase relative to the segmented sample mask. To determine whether the 20 micrometer resolution scan sufficiently captured micro-porosity within the skeleton the 10 micrometer scans were registered to the 20 micrometers scans using the Register Images algorithm of Avizo. The same 12x12x12 mm^3 ROI was then extracted from the 20 micrometer dataset and trends in porosity compared between the two datasets at different resolutions (Supp. Fig. 5).

$$V_{sample} = V_{skeleton} + V_{porosity} \quad (1a)$$

$$V_{porosity} = V_{organic} + V_{pores} \quad (1b)$$

Bulk skeletal density (dry weight of the skeleton / V_{sample}; g/cm^3) and porosity ($V_{porosity}$ / V_{sample} x 100; %) were the two main parameters we derived from downstream analysis of the skeletal architecture dataset. These two parameters are strictly correlated with each other, indeed, as porosity decreases, bulk skeletal density approaches micro-density and neither can exceed the density of pure aragonite (2.94 mg mm^{-3}; Caroselli et al. 2016). The complementarity of the two parameters also has implications on the analytical procedure used to measure them, resulting in multicollinearity in downstream analyses. Therefore, we decided to only retain the parameter porosity as representative of skeletal architecture.

417 Reproductive mode

418 The reproductive mode of each coral species was retrieved from Coral Trait Database (CTDB) v. 1.1.1
419 (https://coraltraits.org/; Madin et al. 2016) and we divided them into three groups: broadcast spawners
420 (*A. aspera*, *G. retiformis*, *G. tenuidens*, *M. digitata*, *P. annae*, *P. australensis*, *P. daedalea*, *P. lutea*, *P.*
421 *sinensis*), brooders (*I. palifera*, *S. pistillata*) and mixed (*P. damicornis*).

422 Statistical analysis

423 The significance level for statistical analyses was 0.05 and unless stated otherwise all analyses were
424 conducted on rarefied ASVs tables (10,000 sequences per sample). Differences in community
425 composition (β-diversity) among coral skeleton, tissue, seawater and sediment microbiomes were
426 computed using center log-transformed Euclidean distance matrices of the rarefied ASV tables.
427 Differences among groups were tested using non-parametric multivariate analysis of variance
428 (NPMANOVA), Linear discriminant analysis Effect Size (LEfSe; Segata et al. 2011) and visualized
429 with principal component analysis (PCA). LEfSe implemented in *microbiomeMarker* (Yang Cao 2020)
430 was used to identify differentially abundant ASVs among groups by coupling statistical tests to assess
431 their differences with further tests encoding biological consistency and effect relevance. ASVs with
432 log(LDA) > 3 (Kruskal-Wallis test: $p < 0.05$) were considered differentially abundant. Z-score
433 transformed abundance profiles of LEfSe identified ASVs were visualized using heatmap via *pheatmap*
434 (Kolde 2012). Associations between the microbiome and predictor variables (host phylogeny, skeletal
435 architecture and reproductive mode) were assessed via variation partitioning analysis (Legendre et al.
436 2005) and canonical correspondence analysis (CCA; Ter Braak 1987), using Hellinger transformed,
437 rarefied ASV tables. CCA is a multivariate method that can disentangle patterns or changes in biological
438 communities and identify potential associations between explanatory variables and ASVs (based on
439 their coordinates' similarity on the CCA plots). Both analyses (variation partitioning and CCA) were
440 performed on the top 200 most abundant ASVs of the dataset, both to reduce its dimension and because
441 rare species may have an unduly large influence on these types of analysis (Legendre and Gallagher
442 2001). All analyses were conducted using RStudio version 1.2.5033 and packages *agricolae* (de
443 Mendiburu and Yaseen 2020), *ampvis2* (Andersen et al. 2018), *ape* (Paradis and Schliep 2019),

decontam (Davis et al. 2018), *dplyr* (Wickham 2015), *ggfortify* (Tang et al. 2016), *ggplot2* (Wickham 2011), *ggvegan* (Simpson 2015), *microbiome* (Lahti and Shetty 2018), *microbiomeMarker* (Yang Cao 2020), *pheatmap* v1.0.12 (Kolde 2012), *phyloseq* (McMurdie and Holmes 2013), *rgr* (Garrett 2013) and *vegan* (Simpson 2015).

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Data availability

Sequence data determined in this study are available at NCBI under SRA Accession PRJNA719930 (https://www. ncbi.nlm.nih.gov/sra/PRJNA719930).

Supplementary Material

Supplementary data, data analysis workflow, raw tables, high quality figures and plots are available at https://figshare.com/search?q=Host+traits+and+evolution+shape+key+coral-bacterial+symbioses

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Figures

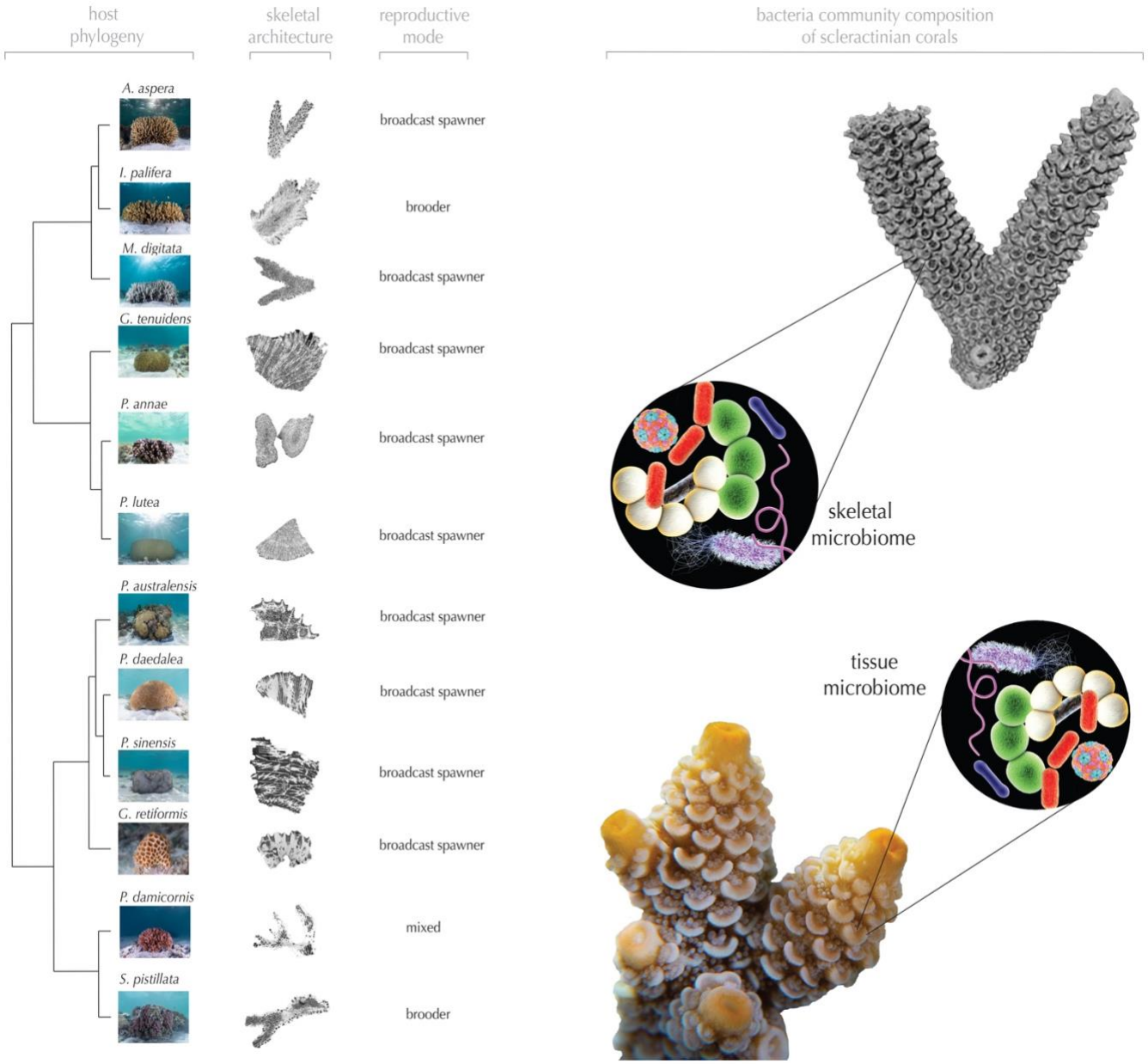


Figure 1. Vignette of the experimental design aiming to answer the question: to which extent host phylogeny, skeletal architecture and reproductive mode affect the microbiome composition of tissue and skeleton of scleractinian corals?

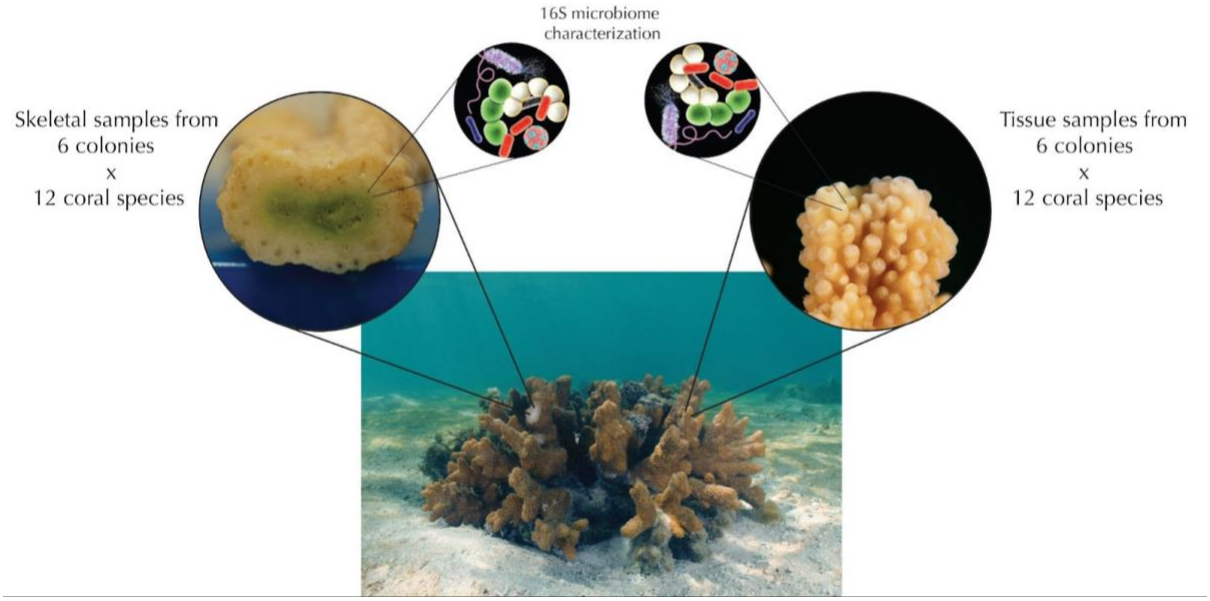


Figure 2. Vignette of the experimental design aiming to answer the questions: what are the microbiome structure of tissue and skeleton of scleractinian corals and how do they differ?

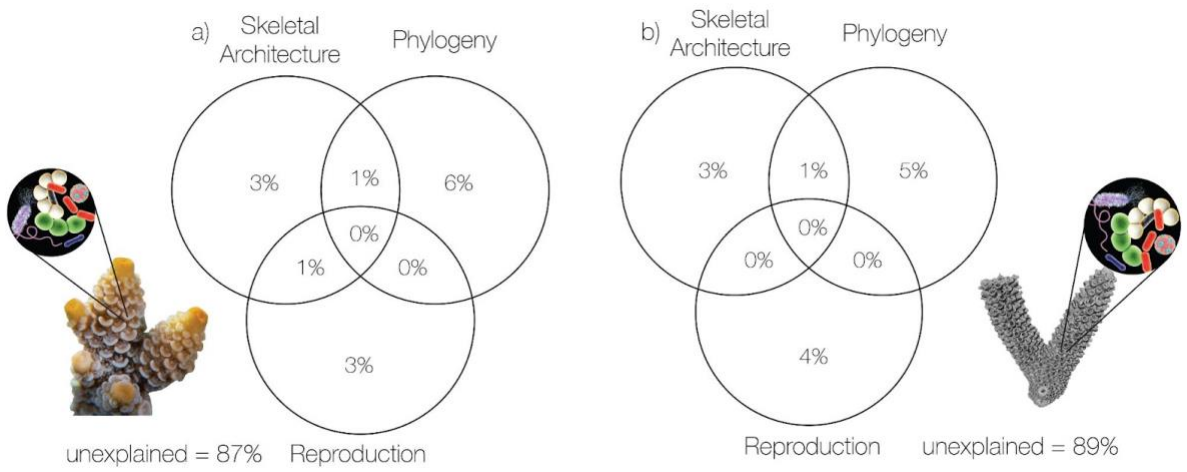


Figure 3. Partitioning the variation in bacterial communities composition of coral tissue (a) and skeleton (b) explained by host skeletal architecture, phylogeny, and reproductive mode. Adjusted R^2 are given.

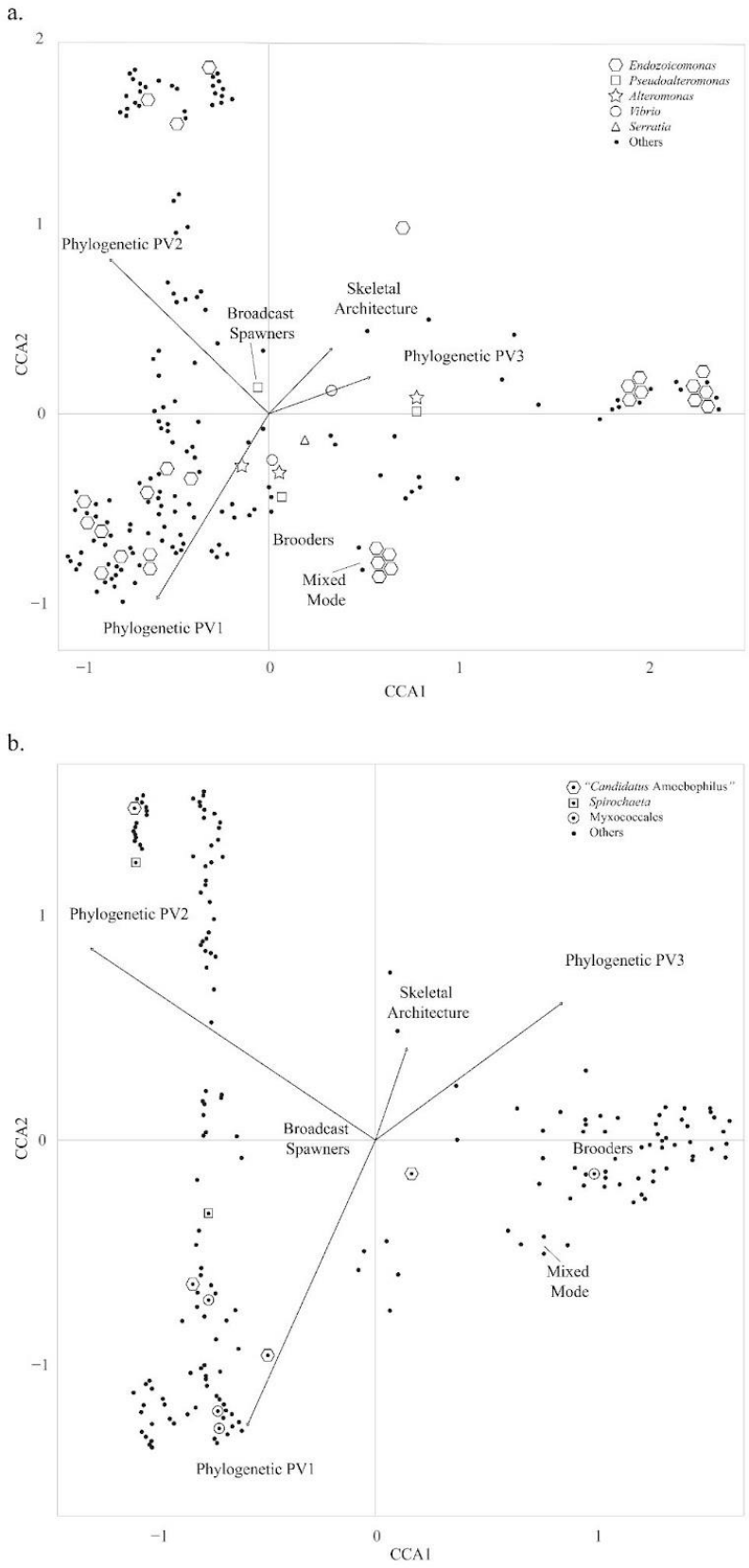


Figure 4. CCA biplot representing the tissue (a) and skeletal (b) microbiome structure according to each explanatory variable. Arrows represent quantitative explanatory variables Skeletal Architecture, Phylogeny PV1, 2, and 3, with arrowheads indicating direction of increase. Categorical explanatory variables Broadcast Spawners, Brooders, and Mixed Mode are positioned on the biplot according to their coordinates. All the explanatory variables in both cca biplots were highly significant ($p < 0.001$).

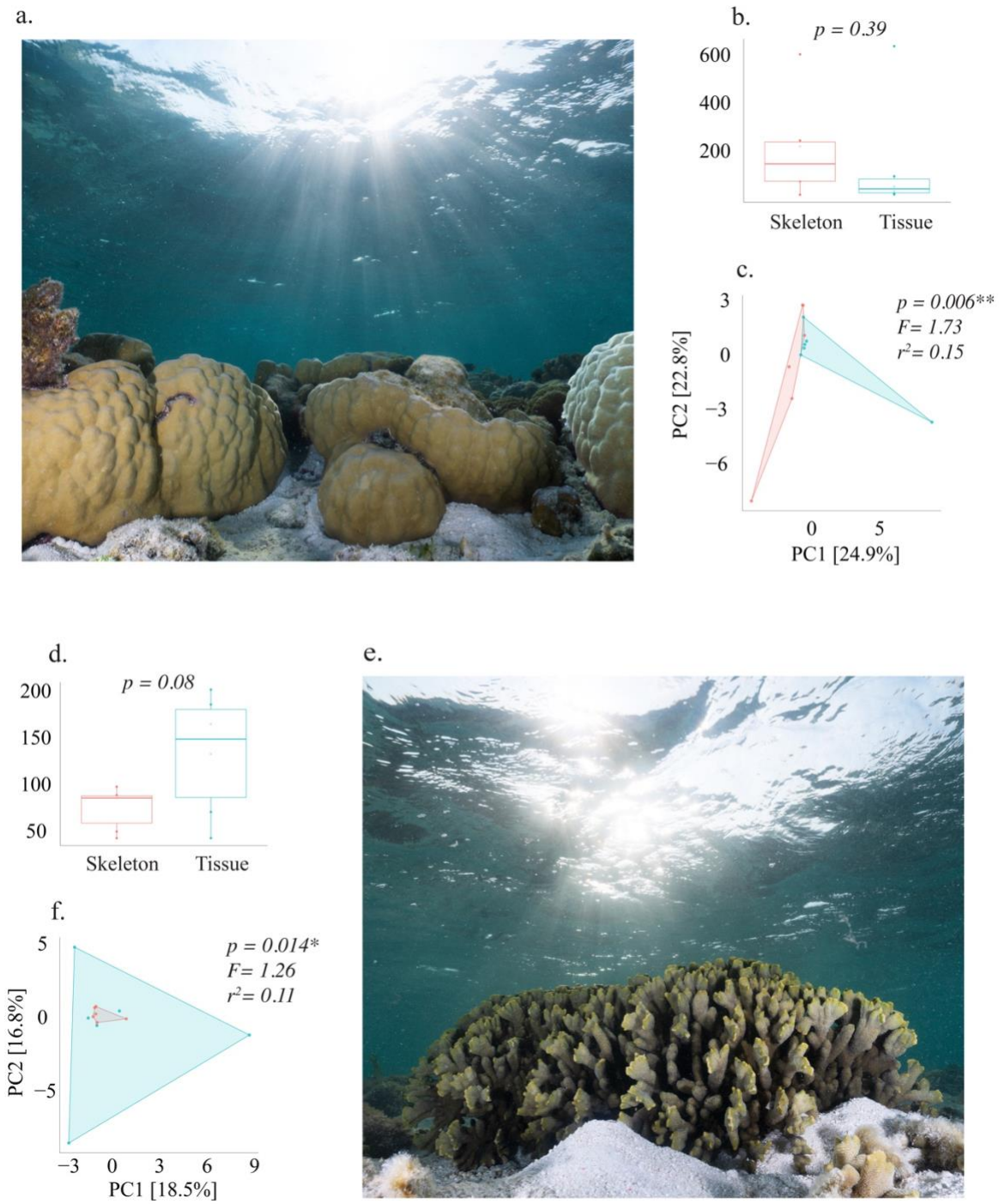
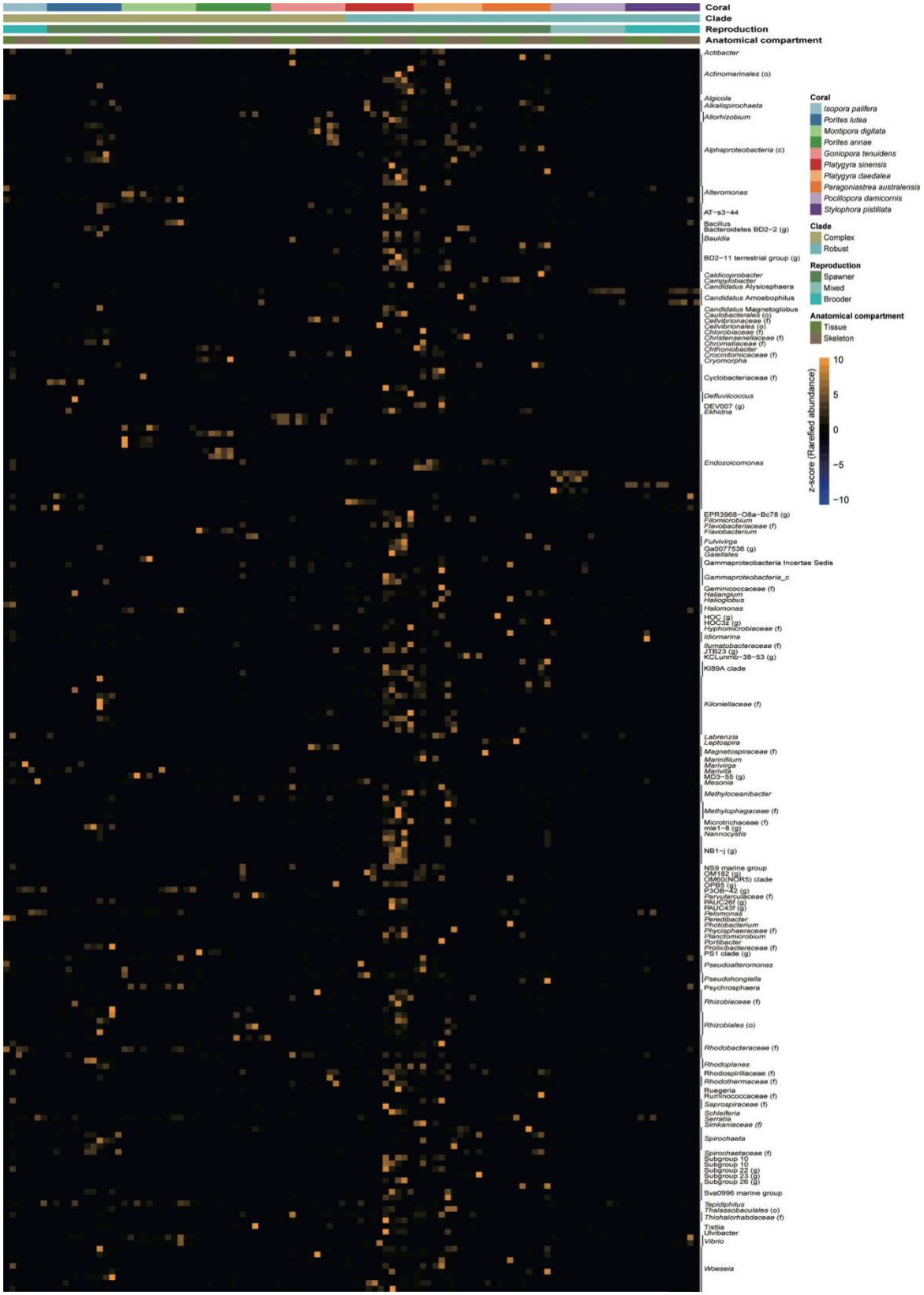
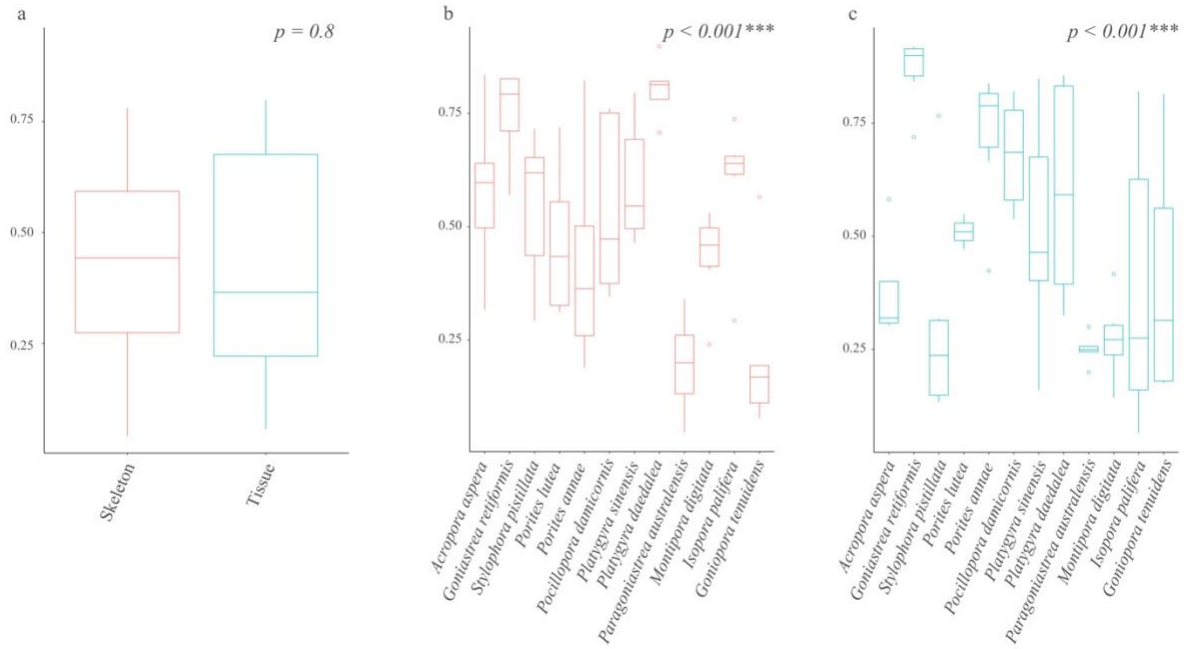


Figure 5. Plots showing differences in microbial communities of *Porites lutea* (a-c), and *Montipora digitata* (d-f). Box-plots b and e show comparisons between observed richness (α-diversity) of tissue and skeleton microbial communities. PCA plots c and f show comparison between tissue (blue) and skeletal (red) microbiome composition and NPMANOVA results. Refer to Supplementary Figure 3 and 4 for additional coral species.

Supplementary Figures



Supplementary Figure 1. Heatmap resulting from the LEfSe analysis showing differentially abundant ASVs between coral skeleton and tissue. In the map ASVs with log(LDA) >3 (Kruskal-Wallis test: $p<0.05$) were considered differentially abundant. Z-score identified ASVs with differentially abundant profiles generated through the LEfSe analysis.



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775 **Supplementary Figure 2.** Boxplots showing Pielou evenness indexes of the skeleton (red) and tissue
776 (blue) of scleractinian corals (a) and each coral species (b and c). Within each plot are reported the *p*-
777 value calculated by means of Mann-Whitney U Test (a), Analysis of Variance (b) and Kruskal–Wallis
778 test (c).

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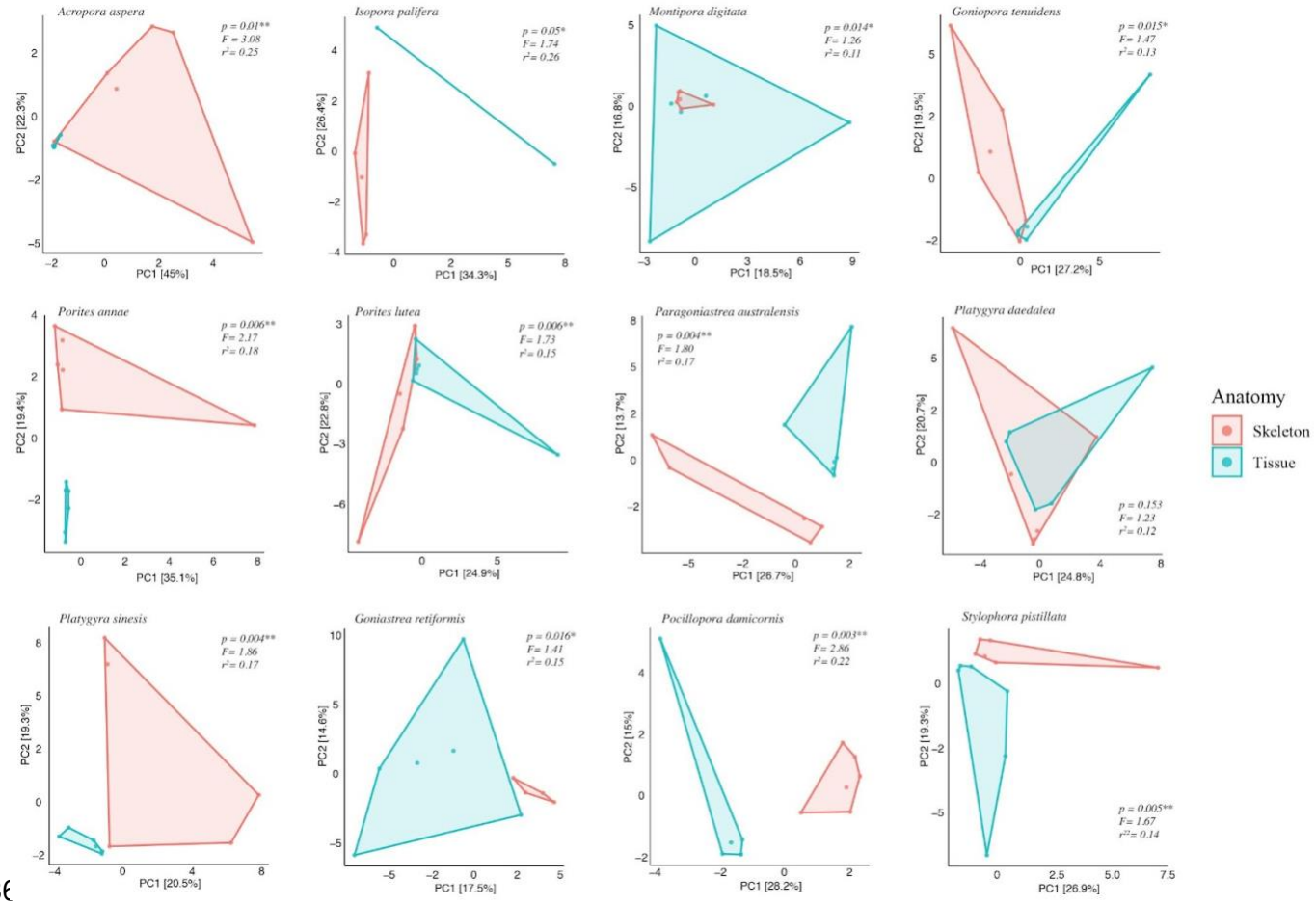

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787

788 **Supplementary Figure 3.** Biplots showing PCA of each coral species. Red dots and areas represent
789 skeletal samples, while blue areas and samples represent tissue samples. Within each biplot are also
790 reported the results of NPMANOVA analyses assessing differences in composition between the
791 prokaryotic communities of skeleton and tissue of each coral species.

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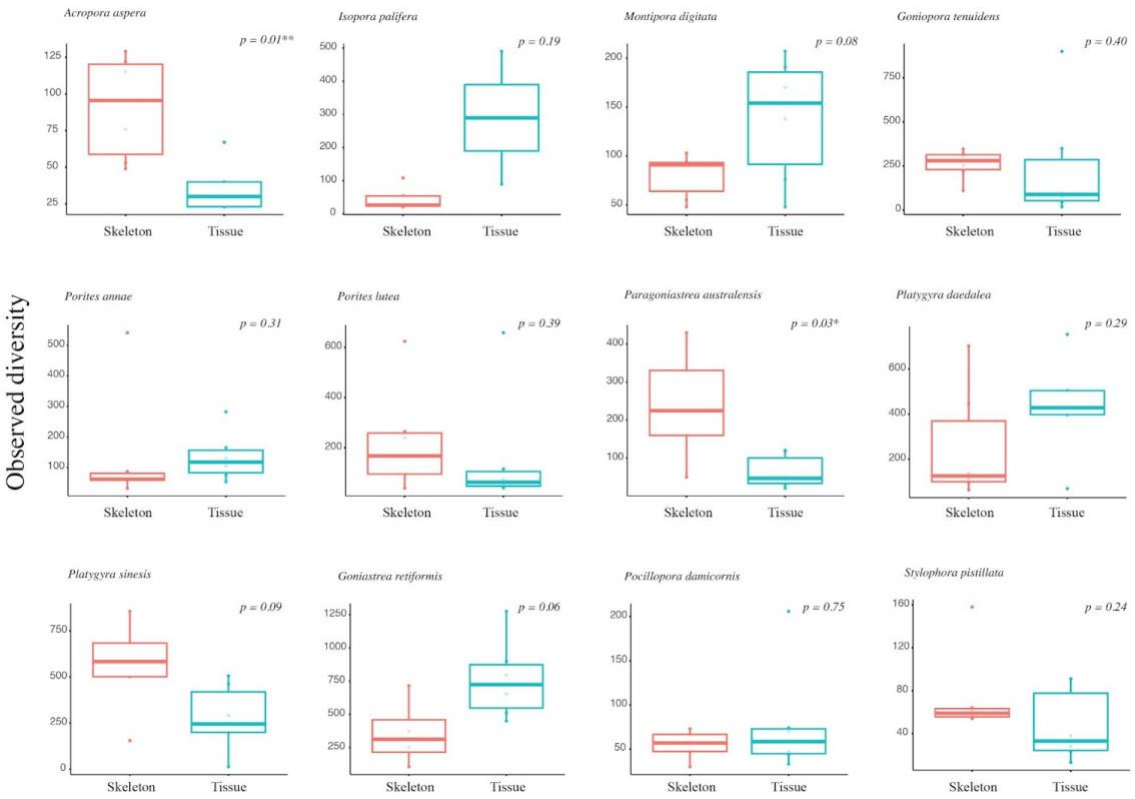
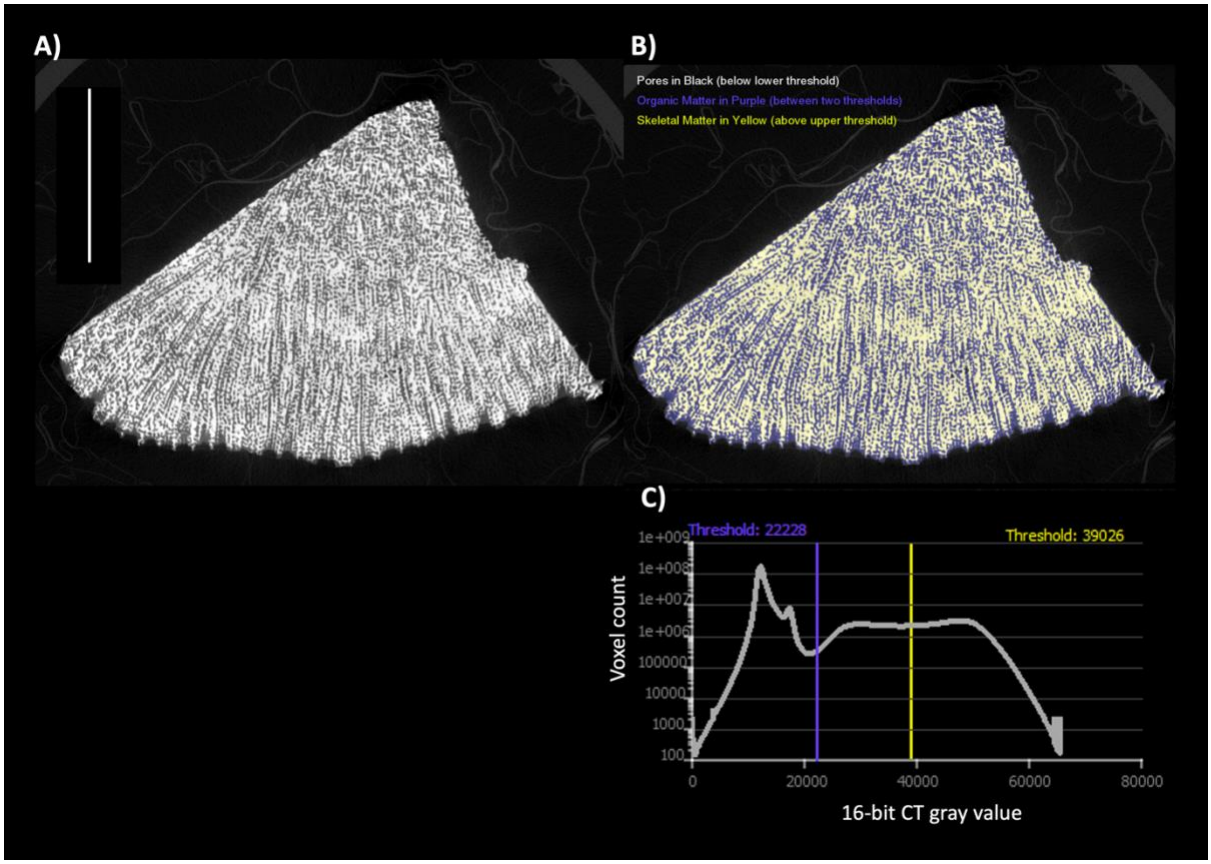
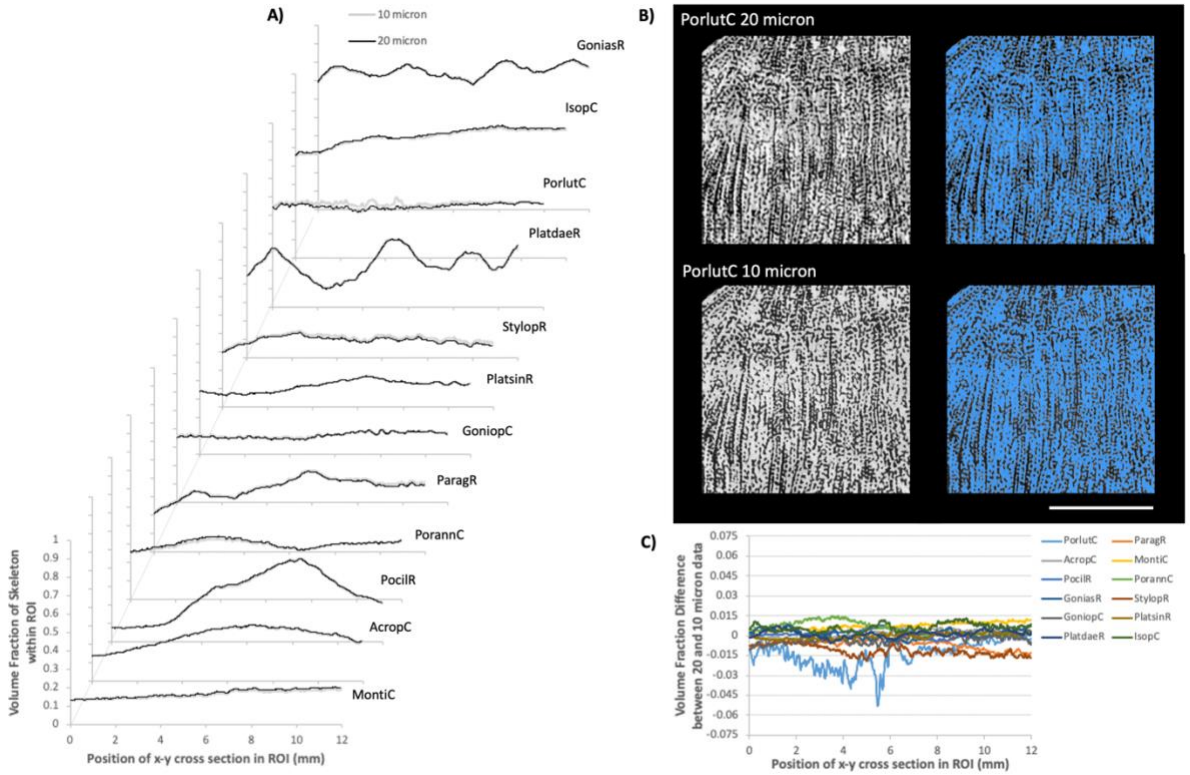


Supplementary Figure 4. Boxplots showing observed α-diversity measures of the tissue (blue) and skeleton (red) of each coral species. Within each plot are reported the *p*-value calculated by means of Welsh T-test or Mann-Whitney U Test.



Supplementary Figure 5. (a) Representative slice of micro-CT data through a sample of *Porites lutea* (FRH49) showing denser structure as brighter gray to white values and less dense structure, such as air, as dark gray to black values surrounding the specimen. (b) Same slice segmented into 3 phases: skeletal material (shaded yellow); organic matrix (shaded purple); and air/bubble wrap within and surrounding the specimen (unshaded gray values). (c) Grayscale histogram of 16-bit micro-CT data showing the points of segmentation between the different phases. The two peaks within the lowest density phase represent the air within and surrounding the specimen (peak around 12000) and bubble wrap and plastic specimen holder (peak around 17000). The white bar in part A is a 10 mm scale bar.



Supplementary Figure 6. (a) Plots of skeletal volume trends within the x-y cross section of a 12x12x12 mm^3 ROI as function of distance along the z-axis of the ROI for both 20 micrometer and 10 micrometer micro-CT data. (b) Representative slice (at 4.3 mm) from sample PorlutC comparing the 20 micrometer to 10 micrometer micro-CT data (left hand figures) and segmented skeleton in both (right hand figures). White scale bar = 6mm. (c) Difference between the 20 micrometer and 10 micrometer data trends plotted in part A highlighting the very small variability within the porosity trends with no consistent trend towards an under- or over- estimate of porosity at one given resolution.

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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SuppData1LeFSe.xlsx
- SuppData2coremicrobiome.xlsx
- SuppData3speciescoremicrobiome.xlsx
- SuppData4n2fixsulfred.xlsx
- SuppData5Xcoralenvironment.xlsx