

Evidence for microbially-mediated tradeoffs between growth and defense throughout coral evolution

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This PDF file includes:

Main Text

Figures 1 to 4

Abstract

Evolutionary tradeoffs between life-history strategies are important in animal evolution. Because microbes can influence multiple aspects of host physiology, including growth rate and susceptibility to disease or stress, changes in animal-microbial symbioses have the potential to mediate life-history tradeoffs. Scleractinian corals provide a biodiverse, data-rich, and ecologically-relevant host system to explore this idea. Using a comparative approach, we tested if coral microbiomes correlate with disease susceptibility across 425 million years of coral evolution by conducting a cross-species coral microbiome survey (the “Global Coral Microbiome Project”) and combining the results with long-term global disease prevalence data. Interpreting these data in their phylogenetic context, we show that microbial dominance predicts disease susceptibility, and traced this dominance-disease association to a single putatively beneficial symbiont, *Endozoicomonas*. *Endozoicomonas* relative abundance in coral tissue explained 30% of variation in disease susceptibility and 60% of variation in microbiome dominance across 40 coral genera, while also correlating strongly with high growth rates. These results demonstrate that the evolution of *Endozoicomonas* symbiosis in corals correlates with both disease prevalence and growth rate, and suggest a mediating role. Exploration of the mechanistic basis for these findings will be important for our understanding of how microbial symbioses influence animal life-history tradeoffs.

65 Main Text

66 Introduction

67 Tradeoffs in life-history strategy are key features in animal evolution^{1,2}. These tradeoffs often
68 involve differential investments in life-history traits such as growth rate³; reproductive maturation,
69 timing, and fecundity⁴; or resistance to stress⁵, predation⁶ or disease⁷. The fitness costs and
70 benefits of these investments are often context-dependent and shifts in ecological or
71 environmental conditions can favor some life-history strategies over others⁵, sculpting trait
72 evolution within animal lineages and reshaping ecological communities. Global climate change is
73 shifting the patterns and prevalence of disease in many animal taxa, while increasing the
74 virulence of some pathogens^{8,9}. Identifying evolutionary tradeoffs and resulting trait correlations
75 associated with disease susceptibility¹⁰ can therefore help predict how species survival will shift
76 with climate change.

77 Although much research on evolutionary tradeoffs focuses on the traits of animals themselves, it
78 is also well documented that the physiology¹¹, fitness and even behavior¹² of many animals are
79 influenced by their microbiomes. Animal microbiomes have been linked to multiple key life-history
80 traits, including growth¹³, development rate¹³, fecundity¹³, stress resistance^{11,14}, and disease
81 susceptibility¹⁴. It therefore seems likely that microbial symbiosis is an important aspect of animal
82 life-history tradeoffs and may correlate with host traits over long periods of animal evolution.
83 However, testing the potential relevance of microbial symbiosis for life-history strategy evolution
84 over long time periods is challenging.

85 The reef-building corals that have evolved over 425 million years represent a diverse group of
86 animals, including an estimated >1600 species¹⁵, with an extensive fossil record, and a well-
87 known variety in both life-history strategy² and microbial symbiosis^{16–18}. As such, they present a
88 valuable opportunity to explore connections between microbes and life history strategy. These
89 animals also have special ecological and societal importance, as corals are foundational to reef
90 ecosystems that support some of the most biodiverse assemblages on the planet and the
91 livelihoods of many coastal communities¹⁹. Yet the ancient diversity of coral reefs is currently
92 threatened by global climate change, which is driving both dramatic mass bleaching events and
93 increased prevalence and severity of disease outbreaks⁸.

94 Alongside research on how coral health is affected by both well-studied (e.g., Symbiodiniaceae^{20–}
95 ²²) and emerging (e.g., coralicolids, fungi²³) microbial eukaryotes, extensive research has
96 demonstrated that present-day communities of coral-associated bacteria and archaea (hereafter
97 'coral microbiomes') play a myriad of roles in host biology that could impact disease susceptibility.
98 These include antimicrobial production²⁴, predation of pathogens²⁵, jamming of quorum-sensing
99 systems²⁶, and passive competition for space and resources. Yet these microbiomes are also
100 influenced by host traits¹⁶, local environmental factors, and ecological context²⁷, including host
101 disease susceptibility patterns within species²⁸. While this supports a connection between
102 present-day coral life-history, microbiome structure and disease susceptibility, these data do not
103 directly allow for statistical testing of evolutionary hypotheses about potential roles of microbial
104 symbiosis in life history tradeoffs.

105 Clarifying whether microbiome structure and coral life-history traits correlate over coral evolution
106 globally will contextualize studies of extant coral symbiosis and disease at local or regional
107 scales. Several lines of research have created a strong foundation on which such comprehensive
108 comparative evolutionary analyses can be built. Coral disease patterns have been intensively
109 researched, and an increasing number of datasets are now openly available²⁹. Well-curated
110 global databases of coral physiological traits³⁰ have been established and mapped to coral life-
111 history strategies². Finally, several large cross-species studies of corals and their microbiomes

have been launched. These advances provide an opportunity to compare host trait data and microbiome structure from across the coral tree of life.

Here, we test whether microbiome structure correlates with two key aspects of coral life history strategy: disease susceptibility and growth rate. To address this question quantitatively, we first characterized the microbiome composition from visibly healthy samples of 40 coral genera using 16S rRNA gene sequencing results from the Global Coral Microbiome Project¹⁶ (Supplementary Data Table 1a), and subsequently combined these data with coral growth rates from the Coral Trait Database³⁰, and genus-level long-term disease prevalence data from several tropical regions around the globe (Fig. 1). These long-term disease datasets included the Florida Reef Resilience Project data (FRRP, <https://frrp.org/>), Hawai'i Coral Disease Database (HICORDIS)²⁹, and new data covering eastern Australia (this study; Supplementary Data Table 1b). With the resulting microbiome structure, coral growth rate, and disease data across a global distribution of coral genera (Supplementary Data Table 1c), we compared these traits using methods that account for phylogenetic correlations using a time-calibrated multi-gene reference tree of corals³¹.

Results

Coral microbiomes are dominated by a small number of bacterial taxa. The microbiome of corals is often dominated by a few highly-abundant taxa that demonstrate species-specificity^{17,18}, though why these highly-abundant microbial taxa differ across coral diversity is unknown. To test this, we first identified a restricted set of dominant bacterial or archaeal taxa in visibly healthy corals retrieved from mucus, tissue, and skeleton samples of 40 coral genera. ('Dominant taxa' were defined as those that are most abundant on average within all samples from a given portion of coral anatomy in a given coral genus.) Thirty-eight of the coral genera were dominated by the bacterial classes α - or γ -proteobacteria, which are known to include common coral associates¹⁷, with further detailed taxonomic resolution revealing that the number of dominant bacterial and archaeal genera across compartments also remained limited (Fig. 2A; Supplementary Data Table 2). For example, only 17 genera of bacteria or archaea accounted for the dominant microbes in the tissue microbiomes of all 40 coral genera (this number excludes 4 unclassified 'genera' that could not be classified to at least the order level). Mucus and skeleton showed similar trends, with only 16 and 25 dominant genera, plus 2 or 4 unclassified genera, respectively. Across coral genera, *Pseudomonas* was most commonly dominant in mucus (31.4% of coral genera), while *Endozoicomonas* was most commonly dominant in tissue (18%) and *Candidatus Amoebophilus* (13.5%) was most commonly dominant in skeleton microbiomes. Currently the influences of microbiome structure and dominance of particular microbial taxa on coral physiology are not yet well understood.

Microbiome dominance correlates with coral disease susceptibility. We visualized the evolution of coral disease susceptibility and multiple measures of microbiome diversity using ancestral state reconstruction (Figs. S1 & S2), then tested whether microbial alpha or beta diversity correlated with disease susceptibility using phylogenetic generalized least squares (PGLS). We found no evidence for an effect of microbiome ecological richness or evenness (considered individually) on disease susceptibility (Supplementary Data Table 3), and limited evidence for an effect of microbiome composition on disease susceptibility (Supplementary Information; Supplementary Data Table 4). However, given that cross-species differences in a limited number of dominant microbes were very notable in the data, we hypothesized that corals with highly abundant bacterial taxa might display more disease vulnerability. To quantify this, ecological dominance among identified ASVs was calculated using Simpson's Index, which estimates the probability that two species drawn from a population belong to the same group, and thereby incorporates aspects of both richness and evenness simultaneously. We correlated Simpson's Index against coral disease prevalence for either all coral samples, or those in mucus, tissue, or skeleton considered individually. In coral tissue, microbiome dominance significantly correlated with disease, explaining roughly 27% of overall variation in disease susceptibility

across coral species (PGLS: $R^2 = 0.27$, $p = 0.0006$, FDR $q = 0.025$; Supplementary Data Table 3a; Fig S1C). No other combination of alpha diversity measure and compartment correlated with disease after accounting for multiple comparisons (Fig. 2B). Thus, microbiome dominance as measured by Simpson's Index was a far stronger predictor of coral disease susceptibility than α -diversity measures that considered either richness or evenness individually. Regionally-specific analysis, which eliminates potential confounders due to the global nature of the comparison, recaptured this dominance-disease relationship (Supplementary Information; Supplementary Data Table 3b). Further testing showed that corals dominated by γ -proteobacteria drove the dominance-disease trend, suggesting a specific microbe (rather than a general ecological feature) might be responsible for this striking correlation (Supplementary Information; Supplementary Table 3c).

The coral symbiont *Endozoicomonas* drives dominance-disease correlations. Bacteria in the genus *Endozoicomonas* are among the most-studied γ -proteobacterial symbionts of corals. In several species *Endozoicomonas* forms prominent aggregates known as CAMAs (coral associated microbial aggregates) in coral tissue³². In species where *Endozoicomonas* is common, it frequently decreases in relative abundance during coral bleaching or disease³³, suggesting a commensal or mutualistic rather than opportunistic relationship with host health. Further, it has previously been observed that the family Endozoicomonadaceae shows by far the strongest signal of cophylogeny with coral hosts among tested bacterial families in coral tissue¹⁶. In the present dataset, *Endozoicomonas* was also the single genus that most typically dominated coral tissue microbiomes (18% of coral genera; Fig. 2A). We therefore tested whether the signal of microbiome dominance on disease susceptibility could be explained by the abundances of dominant taxa, and found that across all corals in our dataset (regardless of whether *Endozoicomonas* was present and/or dominant; $n = 40$ genera), *Endozoicomonas* abundance explained the overwhelming majority of variation in ecological dominance among coral tissue microbiomes (PGLS: $R^2: 0.60$, $p = 6.2 \times 10^{-10}$, FDR $q = 2.5 \times 10^{-9}$; Figs. 2C & 3A; Supplementary Data Table 5a). Further, the relative abundance of *Endozoicomonas* in coral tissue alone explained 30% of variance in overall disease susceptibility (PGLS: $R^2 = 0.30$, $p = 0.0002$, FDR $q = 0.0004$; Fig. 3B; Supplementary Data Table 5a), exceeding the signal from ecological dominance. *Endozoicomonas* remained significantly correlated with disease susceptibility after testing multiple linear models with depth, temperature, extent of turf algae contact, latitude and overall microbiome richness as confounders (Supplementary Data Table 5b & c). Neither commonly opportunistic microbes in corals (Supplementary Data Table 6), nor other dominant microbes (Supplementary Data Table 7) showed similar patterns (Supplementary Information). Thus, our prior results linking ecological dominance and overall disease susceptibility appear to be largely explained by changes in *Endozoicomonas* relative abundance over coral evolution.

***Endozoicomonas* is associated with high growth rates.** *Endozoicomonas* is linked to metabolic benefits to the coral host^{34,35} and experimental studies have shown that decreases in its abundance is typical with disease^{36,37} or other health stressors such as bleaching³³. This suggests that the striking correlation between *Endozoicomonas* and disease is not due to pathogenesis by *Endozoicomonas*, but instead might arise due to opportunity costs (e.g., in innate immunity, permissiveness to CAMA formation, or symbiosis with defensive microbes within coral tissue). If maintenance of high abundances of *Endozoicomonas* has fitness costs, they may be balanced by metabolic benefits, and we should expect that *Endozoicomonas* would be more abundant in corals with life-history strategies that favor traits such as rapid growth.

If symbiosis with *Endozoicomonas* did play a causal role in coral life-history tradeoffs, we hypothesized that we would see a positive correlation between a beneficial coral trait and *Endozoicomonas* that counterbalances the correlation between *Endozoicomonas* and disease. Given that *Endozoicomonas* is thought to be a metabolic mutualist of corals, and it has recently been suggested to facilitate faster coral growth³², growth rate seemed like a likely candidate for a potential benefit explaining the persistence of coral-*Endozoicomonas* associations. Depending on

the mechanism of action, any such *Endozoicomonas* - growth correlations might depend merely on the presence of *Endozoicomonas*, or alternatively on its relative abundance. Using data from the Coral Trait Database (CTDB)³⁰ we tested whether *Endozoicomonas* relative abundance was correlated with growth rate in corals where we detected *Endozoicomonas* (i.e., the effect of relative abundance alone) and in all corals (i.e., the combined effect of presence and abundance). In both cases, we limited this analysis to only corals with replicated growth rate data (≥ 5 replicates in the CTDB).

While the abundance of *Endozoicomonas* was not correlated with growth rate across all coral genera (tissue PGLS: $R^2 = 0.11$, $p = 0.17$, FDR $q = 0.37$; Supplementary Data Table 8a), across coral genera where *Endozoicomonas* was detected and replicated growth rate data were available ($n = 17$ genera), its relative abundance in tissue was strongly correlated with growth rate (tissue PGLS: $R^2 = 0.31$, $p = 0.024$, FDR $q = 0.024$; Supplementary Data Table 8b). Unlike for disease susceptibility, several additional microbes showed anatomically-specific correlations with the growth rate of their coral hosts, including strong positive correlations between growth and uncultured Rhodobacteria in the family Terasakiellaceae (phylum: Proteobacteria) and negative correlations between growth rate and the archaeal genus *Nitrosopumilis* (Supplementary Information; Supplementary Data Table 9; Fig. S3). However, *Endozoicomonas* appears unique in its association with both growth and disease.

These results are consistent with a pattern in which lineage-specific expansions of *Endozoicomonas* and other microbes within coral microbiomes correlate with or potentially contribute to growth rate. Overall, *Endozoicomonas* may in part explain, or at least correlate with, about a third of known growth rate differences between coral genera. Across the coral genera surveyed in our dataset, initial, low-level symbiosis with *Endozoicomonas* does not correlate with growth rate, but subsequent expansions of the abundance of *Endozoicomonas* within coral microbiomes co-occur with both higher average growth rates and greater disease susceptibility.

Endozoicomonas may mediate growth-defense tradeoffs during coral evolution. Having seen that *Endozoicomonas* is correlated with both disease susceptibility and growth-rate in corals, we investigated if these correlations were stronger or weaker than the direct correlation between disease and growth rate in our dataset. Across genera with both growth rate and disease prevalence data, growth and disease susceptibility were positively correlated. However, this correlation had only a modest effect size and was not statistically significant. Thus, in this dataset *Endozoicomonas* showed stronger associations with both growth and disease than these factors showed with one another, regardless of whether the analysis was conducted across all coral genera (tissue PGLS: $R^2 = 0.12$, $p = 0.17$, FDR $q = 0.17$; Supplementary Data Table 10a) or just those where *Endozoicomonas* was present (tissue PGLS: $R^2 = 0.06$, $p = 0.37$, FDR $q = 0.37$; Supplementary Data Table 10b). This suggested that *Endozoicomonas* relative abundance might not merely mark tradeoffs between growth and disease but may play some causal role in one or both processes.

Phylogenetic path analysis of growth, disease, and *Endozoicomonas* abundance. The univariate correlations between *Endozoicomonas*, host disease susceptibility and growth rate raise the question of the direction of causality by which these factors have become non-randomly associated during coral evolution. Using phylogenetic path analysis (Methods), we compared 14 models of the relationship between *Endozoicomonas* relative abundance, disease susceptibility, and growth rate (Supplementary Data Table 11a, Fig. S4).

As is common in this type of analysis, more than one model was consistent with the data. However, none of the top models using either BM (Supplementary Table 11b) or Pagel's lambda (Supplementary Data Table 11c) suggested that disease influenced growth rate or vice versa without the influence of *Endozoicomonas* (Fig. 3D), and all significant models include *Endozoicomonas*. Thus, while the precise feedback remains to be determined, causality analysis suggests that, in some capacity, *Endozoicomonas* likely mediates growth rate and disease.

264 Discussion

265 The findings of positive correlations between *Endozoicomonas*, host growth rate, and host
266 disease susceptibility documented in this study complement and contextualize much of the
267 ongoing work on the mechanisms underlying proposed coral-*Endozoicomonas* metabolic
268 mutualism^{32,34} and suggest that the interaction of *Endozoicomonas* with coral disease
269 susceptibility deserves greater scrutiny. They also echo findings of correlations between life-
270 history strategy and microbiome structure in other important marine invertebrates, such as that
271 between predator defense and microbial abundance in marine sponges³⁸.

272 The mechanism by which corals with high proportions of *Endozoicomonas* become more
273 vulnerable to disease are not yet known, but potential explanations fall into three main categories:
274 ecological, structural, or immunological.

275 Many coral microbes (but not *Endozoicomonas*) are thought to protect against pathogenic
276 disease by mechanisms such as antibiotic secretion²⁴, direct predation²⁵, jamming of quorum
277 signaling²⁶, and through physically occupying space close to host tissues that may restrict binding
278 sites for opportunists and pathogens. In theory, it is possible that *Endozoicomonas* abundance
279 may interact with other aspects of coral microbial ecology, thereby reducing microbially-derived
280 host defenses. However, that *Endozoicomonas* are frequently observed in discrete CAMAs
281 complicates this possibility, as any effects on microbes outside the local area of these CAMAs
282 would have to rely on indirect consequences of *Endozoicomonas*-coral interactions or secreted
283 factors. Nevertheless, if this hypothesis were correct, the reductions in the abundance of
284 *Endozoicomonas* that are often reported in diseased coral phenotypes (e.g.,³³) would then be
285 adaptive on the part of the host, by allowing proportionally greater growth of other, more
286 protective microbes. This hypothesis could be tested by microbial inoculation experiments that
287 increase *Endozoicomonas* abundances prior to or concurrent with disease exposure, with the
288 prediction that this would increase disease severity (although care must be taken to exclude
289 nutritional benefits from corals directly eating the *Endozoicomonas* confounding the results). More
290 systematic studies of whether high abundances of *Endozoicomonas* are exclusively found in
291 visible CAMAs could also speak to the plausibility of this ecological hypothesis, by clarifying the
292 likely routes for interaction between *Endozoicomonas* and other coral-associated microbes.

293 In addition to ecological interactions, the *Endozoicomonas* - disease susceptibility correlation may
294 also arise as a result of host traits that are permissive for the formation of microbial aggregates.
295 As the cellular processes involved in establishing mutualism, commensalism and pathogenesis
296 often overlap, the same host-microbe interactions that allow *Endozoicomonas* and some other
297 microbes like *Simkania*³² to aggregate within coral tissues may also be more permissive towards
298 invasion by pathogens. So far known coral pathogens have not been reported to be present
299 within CAMAs. However, other structural mechanisms are possible. For example, the density,
300 morphology, or diversity of septate junctions — which form epithelial barriers similar to tight
301 junctions in chordates³⁹ — might, in theory, influence the ability of both *Endozoicomonas* and
302 pathogenic microbes to enter coral tissues. This idea could be tested by examining cellular
303 morphology, sequence similarity, and/or gene expression of septate junctions and their
304 constituent components in coral species in which CAMAs did or did not form.

305 Finally, it is possible that coral immunological strategies that permit symbiosis with high
306 abundances of *Endozoicomonas* also tend to make corals more vulnerable to pathogens. Coral
307 species vary in immune investment (as measured by immune parameters like melanin
308 abundance, phenoloxidase activity, etc.), and low immune investment has been observed to
309 correlate with disease susceptibility⁴⁰. Some theory predicts that the evolution of more permissive
310 immunological strategies is favored by symbionts that provide metabolic benefits to the host⁴¹. In
311 corals specifically, immune repertoires in key gene families such as TIR-domain containing genes
312 vary greatly between species, which has been hypothesized to influence microbiome structure⁴².
313 Indeed, in sequenced coral genomes the copy number of some of these, such as IL-1R

receptors, appear to correlate with several features of coral microbiomes, including *Endozoicomonas* abundance⁴³. Thus, symbiosis with *Endozoicomonas* may promote lower immune investment in corals, which in turn increases disease susceptibility. This hypothesis could be tested by comparing the length of coral-*Endozoicomonas* associations, to see whether longer histories of association lead to low immune investment, or by examining selection on innate immune genes in low vs. high *Endozoicomonas* coral lineages (e.g., by dN/dS ratios).

A related immunological explanation would occur if *Endozoicomonas* itself achieves high abundances by suppressing aspects of host immunity. Genomic studies of host-associated *Endozoicomonas* identified variation in the proportion of eukaryote-derived genes and domains as a key feature of strain variation, including some domains thought to suppress immunity-induced apoptosis⁴⁴. If representatives of those different strains could be cultured, experiments adding exogenous *Endozoicomonas* might clarify whether *Endozoicomonas* strains have any direct effects on coral immunity, and if so whether they differ from strain to strain.

Conclusions. Animals evolved in a microbial world. The resulting interactions between animal hosts and their associated microbes influence organismal fitness, and the history of these interactions across generations may influence eco-evolutionary patterns. Using evolutionary analyses of coral microbiomes, we provide evidence that symbiosis with *Endozoicomonas* may mediate growth vs. disease resistance (defensive) tradeoffs. While further manipulative studies are necessary to confirm this finding and determine the directionality of the relationship, evidence for this trend across the coral tree of life is compelling.

Our comparative approach suggests that *Endozoicomonas*-dominated lineages of corals may grow more quickly under ideal conditions but are more likely to succumb to coral disease. Because much other work has shown that coral disease is exacerbated by global and local stressors such as climate-change driven heat waves or local pollution events^{45,46}, this may make *Endozoicomonas*-dominated coral especially vulnerable to environmental change (Fig. 4).

If microbial symbiosis does play a causal role in coral life history tradeoffs in the present day, then identifying microbes underlying those tradeoffs may benefit microbiome manipulation for targeted coral conservation and restoration strategies. For example, microbial screening(e.g.,⁴⁷) could help identify *Endozoicomonas*-dominated coral species or populations that may be more susceptible to disease and drive the conservation and protection of these individuals or their habitats. Identification of these target corals is perhaps most relevant for coral restoration initiatives that include breeding, nursery propagation and out-planting, where coral health is monitored closely and predicting disease susceptibility can inform decision-making. Depending on the mechanism underlying the *Endozoicomonas*-disease susceptibility correlations reported here, *Endozoicomonas*-dominated corals may further represent strong candidates for microbiome engineering (e.g., human-assisted manipulation of host-associated microbes⁴⁸ or the application of probiotics^{14,49}) to enhance host resilience in anticipation of stress events by decreasing microbiome dominance. That said, we emphasize that microbiome manipulation and other restoration initiatives are not replacements for efforts to decarbonize global economies to limit greenhouse gas emissions.

The results presented here provide the first evidence of a likely microbe-mediated life-history tradeoff in Scleractinian corals. Further exploration of this and other such potential tradeoffs may shed light on the evolutionary interplay between microbes and the physiology and ecology of their animal hosts.

Online Methods

Coral sample collection and 16S rRNA pre-processing. 16S rRNA sequence data were obtained from visibly healthy coral DNA extractions collected and processed for the Global Coral Microbiome Project (GCMP). This included coral samples taken from Eastern and Western

Australia that were used in a previous study by Pollock and co-authors¹⁶ in addition to coral samples taken from the Red Sea, Indian Ocean, Coral Triangle, Caribbean, and Eastern Pacific. All samples compared in this study were collected, processed, and sequenced using consistent protocols as outlined below. In total, 1,440 coral, outgroup, and environmental samples were collected. Of these GCMP samples, the 1,283 scleractinian coral and outgroup samples were used in the present study (Supplementary Data Table 1a). These comprise 132 species and 64 genera of corals originating from 42 reefs spanning the Pacific, Indian, and Atlantic oceans.

The collection and processing of these coral samples followed the methods outlined in Pollock et al.¹⁶ and are compatible with samples processed for the Earth Microbiome Project⁵⁰. Briefly, three coral compartments were targeted for each sample: tissue, mucus, and skeleton. Mucus was released through agitation of coral surface using a blunt 10mL syringe for approximately 30 seconds and collected via suction into a cryogenic vial. Small coral fragments were collected by hammer and chisel or bone shears for both tissue and skeleton samples into sterile WhirlPaks (Nasco Sampling, Madison, WI). All samples were frozen in liquid nitrogen on immediate return to the surface prior to processing. In the laboratory, snap frozen coral fragments were washed with sterile seawater and the tissue was separated from skeleton using sterilized pressurized air at between 800-2000 PSI. Tissue and skeleton samples were then preserved in PowerSoil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA; now Qiagen, Venlo, Netherlands) bead tubes, which contain a guanidinium preservative, and stored at -80°C to await further processing. Outgroup non-scleractinian Anthozoans were also opportunistically collected and stored similarly, including healthy samples of the genera *Millepora* (hydrozoan fire coral), *Palythoa* (zoanthid), *Heliopora* (blue coral), *Tubipora* (organ pipe coral), and *Xenia* and *Lobophytum* (soft corals).

Bacterial and archaeal DNA were extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA; now Qiagen, Venlo, Netherlands). To select for the 16S rRNA V4 gene region, polymerase chain reaction (PCR) was performed using the following primers with illumina adapter sequences (underlined) at the 5' ends: 515F⁵¹ 5'- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG YCA GCM GCC GCG GTA A -3' and 806R⁵² 5'- GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT ACN VGG GTW TCT AAT -3'. PCR, library preparation, and sequencing on an Illumina HiSeq (2x125bp) was performed by the EMP⁵⁰. All raw sequencing data and associated metadata for the samples used in this study are available on Qiita (qiita.ucsd.edu) under project ID 10895, prep ID 3439.

Sequence assembly, quality control and taxonomic assignment. 16S rRNA sequencing data were processed in Qiita⁵³ using the standard EMP workflow. Briefly, sequences were demultiplexed based on 12bp Golay barcodes using "split_libraries" with default parameters in QIIME1.9.1⁵⁴ and trimmed to 100bp to remove low quality base pairs. Quality control (e.g., denoising, de-replication and chimera filtering) and identification of amplicon sequence variants (ASVs) were performed on forward reads using deblur 1.1.0⁵⁵ with default parameters. The resulting biom and taxonomy tables were obtained from Qiita (CRC32 id: 8817b8b8 and CRC32 id: ac925c85) and processed using a customized QIIME2 v. 2020.8.0⁵⁶ pipeline in python (github.com/zaneveld/GCMP_global_disease). Taxonomic assignment of ASVs was performed using vsearch⁵⁷ with an extended version of SILVA v. 138⁵⁸ (see below).

Removal of cryptic mitochondrial reads. Coral mitochondrial reads obtained from metaxa2⁵⁹ were added to the SILVA repository to better identify host mitochondrial reads that may be present in the sequencing data⁶⁰. We refer to this expanded taxonomy as "silva_metaxa2" in code. After taxonomic assignment, all mitochondrial and chloroplast reads were removed. The bacterial phylogenetic tree was built using the SATé-enabled phylogenetic placement (SEPP) insertion technique with the q2-fragment-insertion plugin⁶¹ to account for the short-read sequencing data, again using the SILVA v. 138⁵⁸ database as reference taxonomy. The final output from this pipeline consisted of a taxonomy table, ASV feature table and phylogenetic tree that were used for downstream analyses.

Identification of potential contaminants. Potential contaminants from extraction and sequence blanks (n = 103 negative controls) were identified and removed using the decontam package⁶² in R v. 4.0.2⁶³ with a conservative threshold value of 0.5 to ensure all ASVs that were more prevalent in negative controls than samples were removed (n = 662 potential contaminants). The final feature table consisted of a total of 1,383 samples, 195,684 ASVs, and 37,469,008 reads.

Summary of disease data by coral genus. Disease data were gathered from long-term multi-species surveys in the Florida Keys (the Florida Reef Resilience Program (FRRP), <https://frp.org/>), Hawai'i (HICORDIS²⁹), and Australia (this study). Disease counts for Australian corals were collected over a period of 5 years (2009-2013) across 109 reef sites and 65 coral genera (Supplementary Data Table 1b). At each of the 109 reefs, we surveyed coral health using 3 replicate belt transects laid along reef contours at 3-4m depth and approximately 20m apart using globally standardized protocols⁶⁴. Depending on the reef location, belt transects were either 10, 15, or 20m in length by 2m width making the area surveyed at each reef between 60 and 120m². Within each belt transect, we identified each coral colony over 5 cm in diameter to genus and classified it as either healthy (no observable disease lesions) or affected by one or more of six common Indo-Pacific coral diseases (according to Lamb and co-authors⁶⁵). Together with the FRRP and HICORDIS data, the combined disease dataset contained 582,342 coral observations across 99 coral genera (Supplementary Data Table 1c).

Because many of these disease observations identified corals only to genus, disease prevalence data were summarized at the genus level. All three resources represent coral surveys over time, ranging from 5 to 16 years. We chose such long-term datasets in an attempt to minimize the potential effects of specific events (e.g., bleaching in a single summer) and instead to capture more general trends in disease susceptibility across species, if such trends were present. Summarizing these data at the genus level was thus part of a comparative strategy, enabling us to extract overall trends and average out local circumstances, so that we could find holobiont features that control disease resistance that may protect some corals but not others. When summarizing at the genus level, individual counts of healthy corals or corals with specific diseases were summed within coral genera across these datasets.

To ensure sufficient replication, we excluded coral genera with fewer than 100 observed individuals. This minimal count was selected because it is the lowest frequency at which diseases with a reasonably high frequency (e.g., 5%) can be reliably detected. (With 100 counts, there is a >95% chance of detecting at least one count of any disease present with $\geq 5\%$ prevalence; cumulative binomial, 100 trials, success chance = 0.05). Because only very rarely observed taxa were removed, this filtering preserved 99.8% of total observations. Ultimately, our genus-level summary produced a table with 581,311 observations across 60 coral genera (Supplementary Data Table 1d).

Summary of the microbiome data by coral host genus. Statistical summaries of microbiome community composition were calculated for each sample in QIIME2⁵⁶, and then summarized within anatomical compartments and coral genera. These summaries of coral microbiome alpha diversity were richness (observed features per 1000 reads), evenness (the Gini Index), and Simpson's Index, which combines both richness and evenness. Thus, each combination of coral genus and anatomical compartment — such as *Acropora* mucus — was assigned an average α -diversity value.

Simpson's Index, which is of particular importance in these results, is at its highest when a single taxon is the only one present in microbiome, and at its lowest when there are both a large number of taxa, and all taxa have equal abundance. Thus, this measure is reduced both by community richness and community evenness (Simpson's Index is closely related to Simpson's Diversity, which is calculated as $1 - \text{Simpson's Index}$, such that more rich or even communities produce higher values).

Construction of a genus level trait table. The summarized, genus-level disease susceptibility data compiled from all disease projects, and the summarized genus-level microbiome diversity data (see above) were combined to form a trait table that was used in subsequent evolutionary modeling. Additionally, the relative abundance of ‘dominant’ microbes analyzed in this study was averaged within genera and added to this genus-level trait table.

Genus-level summary of a reference coral phylogeny. Starting with a published multigene time-calibrated phylogeny of corals³¹ that we had previously used to demonstrate phyllosymbiosis in corals¹⁶, we randomly selected one representative species per genus to produce a genus level tree. This approach was preferred over several alternatives — such as trimming the tree back to the last common ancestor of each genus and reconstructing trait values — because it required fewer assumptions about the process of trait evolution. As microbiome data were not available for all genera on the coral tree (e.g., temperate deep-sea corals), the tree was further pruned to include only the subset of branches that matched those with microbiome data.

Addition of genus-level coral growth data. To examine the influence of microbiome structure on coral traits, we pulled growth data from the Coral Trait Database³⁰ from all coral genera that matched those with both microbiome and disease data, and were collected using consistent metrics (mm/yr). This resulted in growth rate data from 18 coral genera that were subsequently combined with our genus-level trait table (Supplementary Data Table 1d).

Phylogenetic Correlative Analysis. Shared evolutionary history induces correlations in traits between species that violate the requirement of standard statistical tests that observations must be independent and uncorrelated. Thus, special care must be taken to account for phylogeny in comparative analysis. We first applied Felsenstein’s phylogenetic independent contrasts (PIC) to visualize our cross-genus trait correlations using the phytools R package⁶⁶. This method removes the effect of any shared evolutionary histories by calculating differences in trait values (contrasts) between sister taxa. We next examined the relationships between traits using information-theoretic model selection (that is, comparison of AICc scores) to identify phylogenetic generalized least squares (PGLS) models of evolution that best explained the observed distribution of microbiome α - or β -diversity and disease susceptibility (as continuous evolutionary characters) in extant species. We tested 4 evolutionary models in the caper R package⁶⁷. In the first model, we used PGLS with no branch length transformation (i.e. holding $\lambda, \delta, \kappa = 1$). Thus, this first model is equivalent to PIC. In the next 3 models, we transformed branch lengths on the tree by allowing the model to fit either λ , δ , or κ (see below) using maximum likelihood estimation, while fixing the other 2 parameters at 1. We refer to these 4 models as PGLS, PGLS + λ , PGLS + δ , and PGLS + κ . For detailed explanations of each parameter, please refer to Supplementary Data Table 12. Typically, these models estimated very low λ (~ 0), indicating little or low phylogenetic inertia. Multiple comparisons were accounted for by calculating q values for false discovery rate (FDR) control. Significant relationships between the two traits suggests that they are evolutionarily correlated. All statistics reported represent the best PGLS model results.

Additionally, ancestral state reconstructions of key traits were visualized using the contmap function in the phytools R package⁶⁶, which in turn estimates internal states using fast maximum-likelihood (ML) ancestral state reconstruction as implemented in the fastAnc phytools function.

Phylogenetic causality analysis. Observing that A and B are correlated famously does not guarantee that A causes B. However, non-random correlation between A and B does imply some causal association - though there are many possibilities (A causes B, B causes A, a positive feedback loop exists between A & B, some external factor C causes both A and B, etc.). Path analysis represents hypotheses of causality using directed acyclic graphs, then tests the different strengths of association predicted under different hypotheses of causation to test which are consistent with data. The cross-species nature of these data further necessitated use of phylogenetic path analysis, which also accounts for expected trait correlations among related genera. Hypotheses of the direction of causality between microbiome (specifically

Endozoicomonas), disease, and growth rate were tested using a phylogenetic causality analysis performed in the R package phylopath⁶⁸. This analysis tests the ability of different models to explain correlations in trait data. For example, does selection for a high growth rate in turn drive selection for increased *Endozoicomonas* abundance, which then increases disease susceptibility, or does symbiosis with *Endozoicomonas* itself separately increase disease and growth? Fourteen potential causal models were tested to incorporate all biologically plausible pathways between *Endozoicomonas* abundance, disease susceptibility, and growth rate (Supplementary Data Table 11a; Fig. S4). The top performing causality models according to CICc values (using both Pagel's λ and Brownian Motion models of evolution) were averaged for interpretation and visualization.

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Figures and Tables

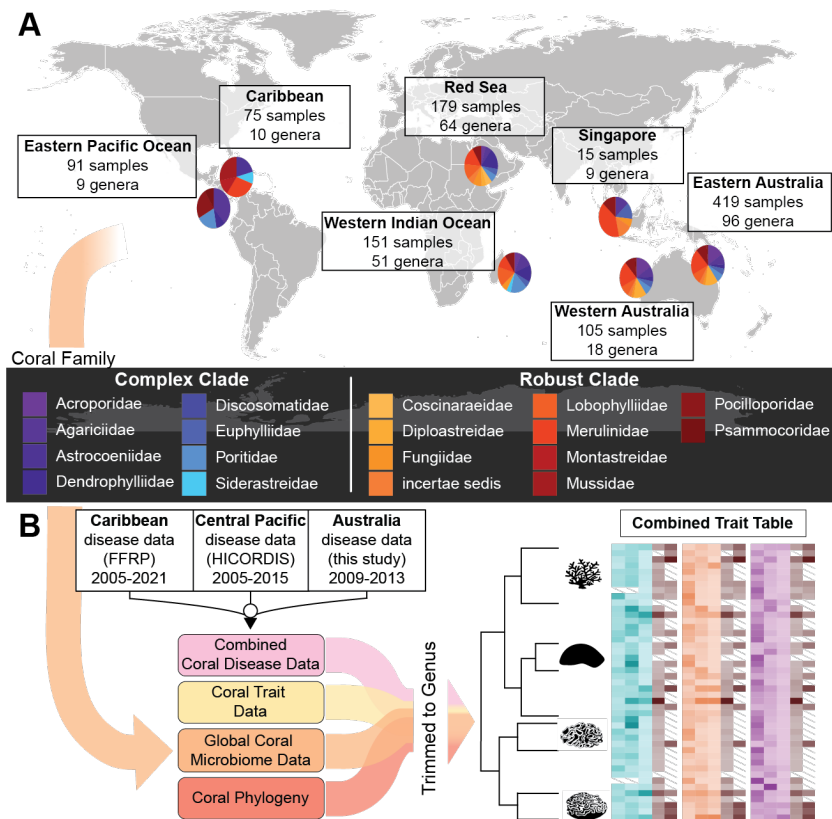


Figure 1. Conceptual overview of data sources integrated for the project. **A.** Map of sampling locations for coral microbiomes analyzed in the manuscript. Pie charts show the proportion of coral samples from families in the Complex clade (cool colors) and Robust clade (warm colors). Samples were collected from coral mucus, tissue, and endolithic skeleton (see Methods). **B.** Schematic representation of data integration for the project. Coral microbiome data (as shown in A) were combined with long-term disease prevalence data from 3 projects (the Florida Reef Resilience Program (FFRP), the Hawai'i Coral Disease Database (HICORDIS), and data from Australia (this study)), as well as coral trait data from the Coral Trait Database, and a molecular phylogeny of corals (see Methods). To integrate data from these disparate sources, all annotations were pooled at the genus level. The end product was a trait table of microbiome, taxonomic, physiological, and disease data across diverse coral genera.

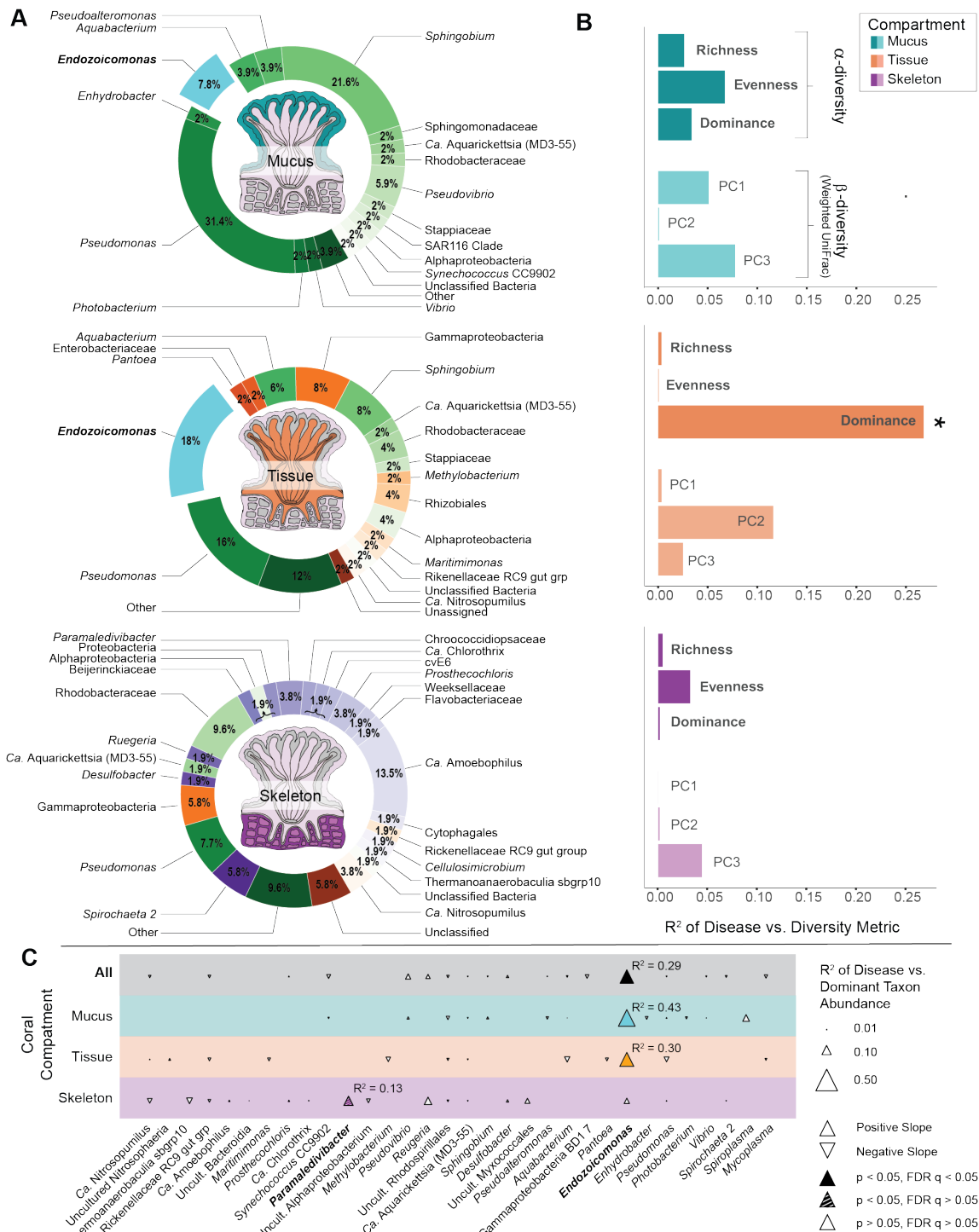


Figure 2. Dominant microbes in the coral microbiome. A. Dominant bacterial or archaeal genera in coral mucus (cyan), tissue (orange), or skeleton (purple) microbiomes. Pie wedges represent the fraction of coral host genera in which the labeled bacterium is more abundant than all other bacterial or archaeal taxa. Cyan shades represent microbes dominant in mucus, oranges represent microbes dominant in tissue (but not mucus), purple shades represent microbes dominant in skeleton (but not mucus or tissue). *Endozoicomonas*, which is of special significance later in the paper, is highlighted in aqua. **B.** Bar charts showing correlations between microbiome

alpha and beta diversity metrics and disease, represented by the R^2 for PGLS correlations. Alpha diversity metrics include richness, evenness (Gini index), and dominance (Simpson's index), and weighted UniFrac beta diversity metrics including the three principal component axes (PC1, PC2, PC3) that represent measures of community structure. Significant relationships ($p < 0.05$, Supplementary Data Table 4) are marked by an asterisk (*). **C.** Bubble plot showing correlations between dominant microbial taxa and coral disease prevalence. The size of each triangle represents the R^2 for PGLS correlations between disease susceptibility and microbial relative abundance for each listed taxon in either all samples (top row), mucus samples (cyan row), tissue samples (orange row), or skeleton samples (purple row). Colored points were significant ($p < 0.05$, FDR $q < 0.05$) and hashed points were nominally significant ($p < 0.05$, FDR $q > 0.05$; Supplementary Data Table 7a). Points that were not significant or had too little data ($n < 5$) for reliable testing are marked in white. Taxa whose abundance is significantly correlated with disease are marked in bold on the x-axis.

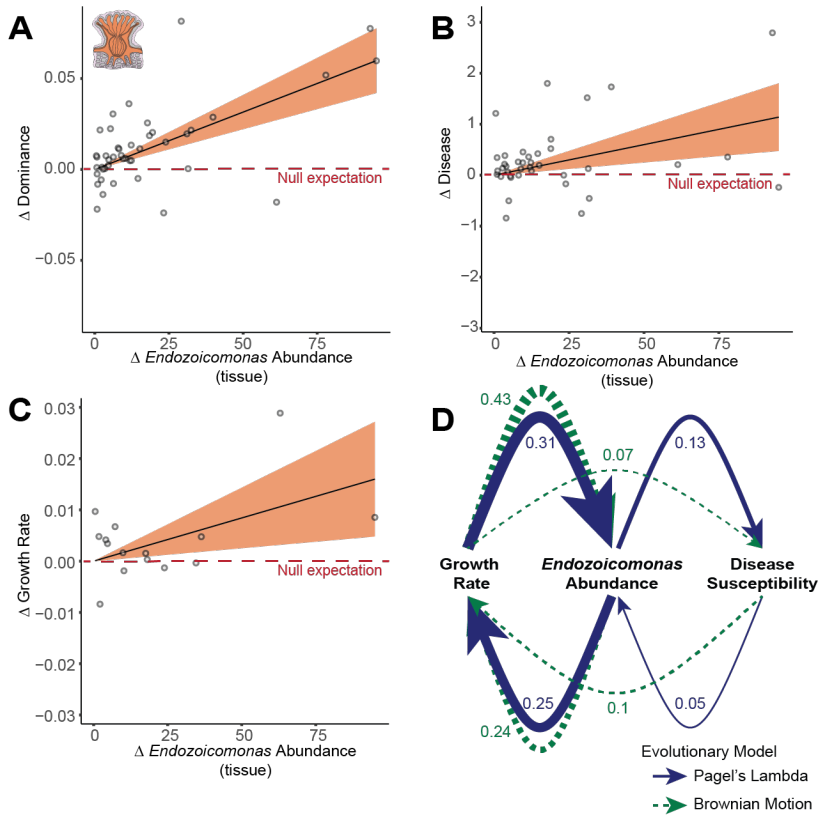


Figure 3. *Endozoicomonas* correlates with growth and disease. Phylogenetic independent contrast in *Endozoicomonas* relative abundance in coral tissue (per 1000 reads), correlated against **A.** contrast in microbial dominance in coral tissue (assessed by Simpson's Index), **B.** contrast in coral disease susceptibility (estimated from integrated long-term coral disease prevalence data) and **C.** coral growth rate (mm per year) from the Coral Traits Database. Dotted red lines in panels A-C indicate the null expectation that if traits are uncorrelated, change in the x-axis trait will not correlate with changes in the y-axis trait, with contrasts instead distributed equally above or below the dotted line. Statistics from phylogenetic generalized least squares (PGLS) regression for A-C are available in Supplemental Data Tables 5 and 9. **D)** Modeled strength and direction of causality between *Endozoicomonas* abundance, disease susceptibility and growth rate during coral evolution using both Brownian Motion (blue) and Pagel's Lambda (green, dotted) evolutionary models. The thickness of the lines represents the averaged standardized path coefficients of the top competing models based on CICc values (Supplementary Data Table 12).

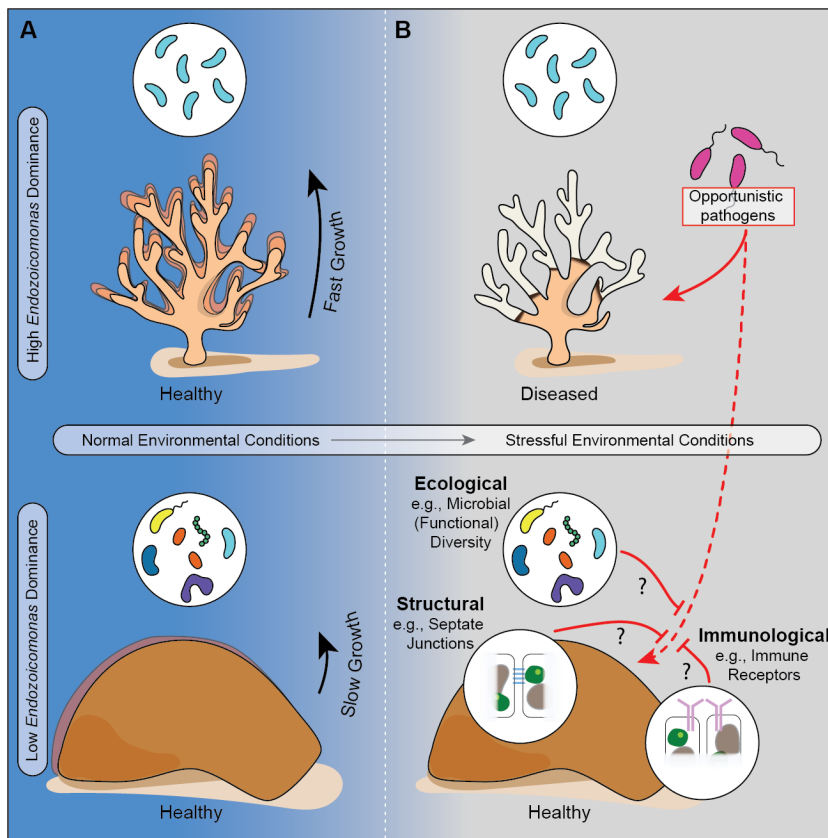


Figure 4. *Endozoicomonas* dominance facilitates life history tradeoffs. Conceptual hypothesis on the role *Endozoicomonas* dominance in coral microbiomes (teal icons, top row) plays in the tradeoff between growth and defense under varying environmental conditions. *Endozoicomonas*-dominated microbiomes may A) provide a metabolic advantage for growth under normal environmental conditions (top left), but B) lack the ecological, structural or immunological defenses against pathogen invasion, and therefore become susceptible to disease under stressful environmental conditions (top right). In contrast, microbiomes not dominated by *Endozoicomonas* (bottom left) grow slower, but may have lower disease susceptibility in stressful environmental conditions (bottom right).