Endozoicomonas provides corals with steroid hormones during thermal stress

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Summary Paragraph

Rising temperatures are devastating coral populations throughout the globe[1]. The coral microbiome is believed to play a critical role in sustaining corals and enabling their adaptation to environmental changes, particularly thermal stress[2–4]. A ubiquitous group of coral-associated bacteria, known as *Endozoicomonas*, are hypothesized to provide their host with essential metabolites[5]. However, the nature of coral-*Endozoicomonas* symbiosis and the role these bacteria play in adaptation to thermal stress are largely unknown. Here, we show that symbiotic *Endozoicomonas* adapt to the host environment by gaining the ability to degrade coral-derived steroids and *Symbiodiniaceae*-derived galactose while losing the ability to synthesize iron-binding siderophores, a common feature of free-living marine bacteria[6]. More importantly, under thermal stress *Endozoicomonas* utilizes coral-derived cholesterol partially as a carbon source while simultaneously converting it to the hormones testosterone and progesterone. Both steroids prime the innate immune system and inhibit pathogenic bacteria and fungi[7]. These findings highlight an unknown interaction between corals and their microbiome that may be critical to coral health as oceans warm up. The ability of bacteria to synthesize eukaryotic steroid hormones underscores the importance of these molecules in inter-kingdom interactions and suggests that their origin may have evolved as a result of eukaryogenesis.

Keywords: coral symbiosis, *Endozoicomonas*, heat stress, chemical diversity, steroids.

Introduction

Corals form intricate associations with a diverse microbiome, collectively referred to as the coral holobiont[8]. This microbiome has been recognized as an essential contributor to coral health and resilience, especially during thermal stress[2–4]. While the symbiotic interactions between corals and their primary algal endosymbiont, Symbiodiniaceae, have been extensively studied[9–11], the functional roles of bacteria in the coral holobiont remain largely unexplored[2,12] despite their importance for coral health[3,13,14]. Among the myriad of bacteria associated with corals, the genus *Endozoicomonas* has received significant attention due to its ubiquitous distribution across coral species and geographic locations[15–17] and its correlation with coral health[15,18,19].

Based on genomic predictions, *Endozoicomonas* spp. are hypothesized to play a crucial role in coral holobiont metabolism through recycling essential nutrients and biosynthesis of B vitamins, amino acids and DMSP[15,20–22]. Indeed, *E. marisrubri* responds to coral tissue extract additions with activation of vitamins B_1 and B_6 biosynthesis and glycolytic processes[5]. However, despite recent efforts[5,22–25], a definitive role for *Endozoicomonas* within the coral holobiont and the precise function underlying its symbiotic association with corals across spatial and taxonomic scales are yet to be identified. In this study, we use metagenomics, metabolomics and steroid addition experiments to show that *Endozoicomonas* provides the coral holobiont with the hormones progesterone and testosterone likely to mitigate effects of thermal stress, a unique role *Endozoicomonas* carries out among all holobiont members.

Results

We monitored and sampled colonies of the coral *Acropora pharaonis* for shotgun metagenomics and metabolomics from the Persian/Arabian Gulf, one of the hottest bodies of water on Earth during summer. Sampling occurred across a temperature gradient starting with early thermal stress and early signs of white syndrome disease at the stress threshold temperature of 32°C (June), severe thermal stress and further white syndrome progression at 34°C (August), and recovery at 27°C (October) (Fig.1a, Extended Data Table 1). Samples were collected from healthy coral tissue at all three temperatures, and from white-syndrome lesion sites (L) and healthy appearing tissue lying adjacent to the lesion (AL) when disease was apparent at 32°C or 34°C (Fig. 1a). All samples were extracted for metabolites and DNA.

The intracellular metabolites of the coral holobiont were extracted and analyzed with reverse phase liquid chromatography-high resolution mass spectrometry (RP-LC/HRMS) in positive and

negative ionization modes and in tandem with MS^2 mass fragmentation. Nearly 3500 putative molecules were predicted based on *in-silico* mass fragmentation network analysis (*see* Methods) and then grouped into chemical classes (Supplementary Table 1, Extended Data Fig. 1a). Principal component analysis (PCA) patterns were significant (adj. *p*-value=0.019) and showed that early stress (32°C) and recovery (27°C) metabolomes clustered together away from severe stress (34°C), suggesting that despite being separated by four months the holobiont metabolic state during early stress and recovery were similar. The metabolomes of the diseased samples (L, AL) displayed intercolony variation and showed a significant separation from all other samples, suggesting a fundamentally different chemical composition compared to healthy tissue (Fig. 1c, Extended Data Fig. 1b). These findings suggest that corals in the Persian/Arabian Gulf exhibit a notable tolerance to higher temperatures, up to 32°C, while exposure to 34°C poses a critical challenge to their overall fitness, which aligns with previous reports[26]. Using an in-house chemical library and commercial spectral libraries, we confirmed the annotation of 300 metabolites, which were used for downstream analyses (Supplementary Table 2).

The metagenomes of the coral holobionts produced a total of 589 gigabases (Gb), averaging 42Gb per sample (Supplementary Table 3). We leveraged publicly available genomes for *Acropora* and *Cladocopium* to partition the holobiont metagenome before assembling coral and algal contigs (*see* Methods). We obtained a total of 257 million reads (~7% of the holobiont reads) as non-coral, non-algal reads (Fig. 1b). These putative microbial reads were used for downstream read-based and assembly-based metagenomic analyses (Extended Data Fig. 2, Supplementary Table 3).

Taxonomic profiling of putative microbial reads revealed a diverse coral microbiome primarily dominated by bacteria (95% of the microbial community; Extended Data Fig. 3a). Interestingly, the bacterial composition of AL and L samples differed significantly from all other samples (PERMANOVA; R-squared: 0.723; *p*-value < 0.001), suggesting the holobiont microbiome undergoes a major shift during disease progression (Fig. 1d). Consistent with this observation, bacterial diversity showed a stepwise increase both in richness and biodiversity with higher temperatures, reaching its peak during disease progression, indicating proliferation of opportunistic microbes during thermal stress and disease onset, such as Rhodobacteraceae (Extended Data Fig. 3b,c)[27–29].

We attempted to distinguish metabolic potential across members of the coral holobiont using an integrated analysis of the metagenomics and metabolomics datasets. To achieve this, metagenomic reads assigned to host, algal symbiont and bacteria were assembled and

functionally annotated to acquire a total of 11,333 KOs. While a third of the predicted KOs (3,065) were shared between different members of the holobiont, most (4,619) were uniquely assigned to the coral host, followed by the microbiome (3,029) (Fig. 1e). Using these KO taxonomic assignments, we mapped the 300 confirmed metabolites (Supplementary Table 2) from the metabolomic datasets to their respective KOs. This analysis indicated 192 of confirmed metabolites were shared between different members of the holobiont, while 39 were assigned uniquely to the host, 22 to *Cladocopium* and 48 to microbes (Fig. 1e, Extended Data Table 2). Examining the KEGG pathways across metagenomes and metabolomes indicated that the host dominates the biosynthesis of steroid hormones, retinol metabolism and vitamin digestion and absorption, while bacteria dominate steroid degradation, biosynthesis of cofactors and biosynthesis of secondary metabolites (Fig. 1e).

Considering the prominence of *Endozoicomonas* within the coral holobiont, we attempted to assemble metagenomically assembled genomes (MAGs) obtained from our metagenomes since culturing efforts of *Endozoicomonas* from *A. pharaonis* were not successful. Coral samples devoid of mucus and seawater yielded 11 high-quality MAGs (completeness >75% and contamination <10%), of which one was designated MAG10 and assigned as *Endozoicomonas acroporae* (Extended Data Fig. 4; Supplementary Table 4). MAG10 represented a core member of the microbiome as it was consistently present at relatively high abundance relative to all other MAGs in healthy tissue at all temperatures with decreasing abundance in AL and L samples. Its relative abundance peaked at 32°C during early stress, followed by a dramatic decrease at 34°C and a modest recovery at 27°C (Fig. 2a).

We examined functional conservation and specialization across *Endozoicomonas* genomes by constructing a pangenome of MAG10 and 27 published *Endozoicomonas* genomes/MAGs from diverse invertebrate hosts (including *Acropora* spp.) and the closest free-living isolate from sediment, *Spartinivicinus marinus* SM1973 (thereafter SM1973) (Fig. 2b, Supplementary Table 5)[25]. Since intracellular symbiont genomes undergo reduction over evolutionary timescales, we expect symbiotic *Endozoicomonas* genomes to be reduced compared to their closest free-living relative. The genome sizes of *Endozoicomonas* symbiotic with invertebrates, including soft corals, undergo a small reduction from 6.8 to 6.2±0.2 Mb, while those symbiotic with hard corals undergo a more drastic reduction to 5.13±1.3 Mb (Supplementary Table 5). These observations suggest a facultative lifestyle for *Endozoicomonas* in invertebrates, supporting previous reports and confirming the absence of genome streamlining (characterized by dramatic genome reduction in obligate bacterial symbionts) within the *Endozoicomonas* genus[15].

We hypothesized that *Endozoicomonas* genomes symbiotic with corals possess a markedly different functional profile compared to SM1973 that enable coral-associated *Endozoicomonas* to adapt to their new host environment. As reported for other genomes of coral-associated *Endozoicomonas* spp., MAG10 harbors genes related to symbiosis establishment, e.g., flagellar assembly, type IV pili[30], ankyrin and tetratricopeptide repeats[5,22], several secretion systems involved in host infection, e.g., types II, III, and VI (T2SS, T3SS, T6SS)[5,22,23,30], the genes required for the biosynthesis of the vitamins riboflavin (B_2), biotin (B_7) and pyridoxine (B_6), all nine essential amino acids and putatively transporting them to the coral host (Extended Data Fig. 4bc, Supplementary Table 6)[15]. MAG10 was also significantly enriched within the holobiont in production of antioxidants[5], heme biosynthesis[25], glycoside hydrolases that cleave the most abundant marine polymers starch and chitin[31], and diverse ABC transporters (Supplementary Tables 6 and 8). In addition to these previously known functions, MAG10 is capable of **(a)** histidine degradation to glutamate, an essential intermediate for ammonium assimilation and a common feature of most *Endozoicomonas* genomes, **(b)** degrading galactose (Leloir pathway), a Symbiodiniaceae photosynthate[32], and a common feature in most *Endozoicomonas* genomes, **(c)** biosynthesizing the nucleoside antibiotic showdomycin, **(d)** biosynthesizing the polyamine spermidine, a reactive oxygen species scavenger, and export proteins for spermidine and its precursor putrescine **(e)** and biosynthesizing ectoine, a bacterial osmoprotectant (Fig. 2b, Extended Data Fig. 4c, Extended Data Tables 3 and 4, Supplementary Table 6). Cumulatively, these functions may enhance symbiosis and increase the coral host and/or Symbiodiniaceae fitness especially during thermal stress.

In contrast, the genome of the closest free-living relative SM1973 contained sixteen unique biosynthetic gene clusters (BGCs), most of which belonged to non-ribosomal peptide synthetases (NRPSs; n=9) that promote the synthesis of diverse natural products[33]. The genome of SM1973 exclusively harbored clusters responsible for the production of the antimicrobial compounds cyanobactin and phenazine and the dipeptide N-acetylglutaminylglutamine amide (NAGGN), an endogenous osmolyte identified in a rhizobial symbiont[34]. The apparent reduction in BGCs in symbiotic *Endozoicomonas* genomes suggests an adaptation to the endogenous host environment, where chemical warfare with other microbes are accessory functions[35]. Surprisingly, SM1973 and MAG10 possessed genes for the biosynthesis of the iron-chelating siderophore aerobactin (Fig. 2b, Extended Data Fig. 4c, Extended Data Table 3).

Siderophores are small molecules secreted by some bacteria to acquire iron in iron-limited environments[6] and have been shown to play important roles in iron cycling and bioavailability in

the pelagic ocean[36,37] with the potential of benefiting corals[38], although notably siderophores have not been detected in corals. The genome of SM1973 possesses the complete genes for aerobactin synthase (*iucA/iucC*) and the TonB-dependent uptake receptor (*iutA*) used to produce apo-aerobactin and take up Fe-bound to aerobactin, respectively. In contrast, MAG10, all *E. acroporae* and 8 other coral- and invertebrate-associated *Endozoicomonas* genomes possessed only the biosynthesis genes while having lost *iutA* (Fig. 3a, Extended Data Table 4). Because *iutA* is required for a functional aerobactin-based Fe uptake system, we hypothesized that coral-associated *Endozoicomonas* do not utilize siderophores inside the host and that the canonical biosynthesis gene *iucA/iucC* may have undergone genetic drift that led to loss of function. A phylogenetic tree of the *iucA/iucC* genes in symbiotic coral-associated *Endozoicomonas* spp., SM1973, and *K. pneumoniae* as a confirmed aerobactin producer, was constructed. *IucA/iucC* from SM1973 clustered with *K. pneumoniae* away from coral-associated *Endozoicomonas*, suggesting significant amino acid mutations differentiate coral-associated *iucA/iucC* (Fig. 3b). To confirm a putative loss of function, we grew *E. acroporae* AT381, originally isolated from the coral *A. kenti*[39] *formerly A. tenuis*, under Fe-limiting conditions and tested the cell-free supernatant and cell pellets for siderophore production using the chrome azurol S assay (CAS)[40]. The CAS assay indicated that *E. acroporae* AT381 had lost its ability to produce aerobactin (Fig. 3c). These findings suggest that MAG10 and other coral-associated *Endozoicomonas* do not use siderophores inside the host and instead utilize other pathways to acquire iron, like heme transporters (Supplementary Table 6).

MAG10 was the only holobiont member that possessed the complete pathway to acquire carbon from the degradation of the steroid hormone testosterone via 3-oxosteroid 1-dehydrogenase (*kstD*) (Fig. 1e; Supplementary Table 6), a function shared with most *Endozoicomonas* genomes (Extended Data Table 4), suggesting a potential reliance on the coral host for growth[41]. Most steroid abundances showed a gradual and consistent decrease as a function of increasing temperatures, with abundance at 34°C, AL and L near background levels (Fig. 4a, Extended Data Fig. 5), suggesting the coral downregulates genes related to steroid hormone biosynthesis under stress and during infection, consistent with previous reports[42]. Surprisingly, a notable exception to this pattern were select key coral steroids and steroid degradation products. Specifically, coral-derived progesterone, testosterone and estradiol-17β, in addition to *Endozoicomonas*-derived androsta-1,4-diene-3,17-dione, 9α-hydroxyandrosta- 1,4-diene-3,17-dione and HIP showed the highest relative abundance at 34°C with most other samples at near background levels (Fig. 4a, Extended Data Fig. 5). Since the sampling timepoints fell outside the spawning season (March to May)[43], the elevated levels of these steroid hormones during severe

stress at 34°C were unexpected. Therefore, we hypothesized that *Endozoicomonas* can manipulate steroid hormone stocks in corals by synthesizing some of the hormones exhibiting elevated abundance during severe stress.

To examine this, cultures of *E. acroporae* AT381 were grown at 34°C in minimal media with or without the steroids cholesterol (the precursor of steroid hormones and one of the most abundant steroid molecules in the coral holobiont)[44], cholestenone (a cholesterol derivative) or testosterone. After 24 hours, *E. acroporae* AT381 utilized all three substrates and accumulated significant amounts of several steroid hormones as well as degradation products of testosterone, similar to observed results in holobiont samples (Fig. 4a). Specifically, *E. acroporae* converted cholesterol and cholestenone into the terminal products progesterone, testosterone and the metabolites HHD and HIP that feed into the TCA cycle (Fig. 4bc, Extended Data Fig. 6). Using high-resolution mass spectrometry and fragmentation information, we propose a pathway that bifurcates cholesterol into progesterone and androstenedione via cholestenone and dihydroxycholesterol, respectively. Androstenedione is subsequently converted into testosterone and siphoned into the TCA cycle via secophenol (Fig. 4bc; Supplementary Table 7). These findings indicate that *E. acroporae* is using cholesterol as both a substrate for synthesizing the key steroid hormones progesterone and testosterone in addition to its use as a carbon source.

Discussion

The cumulative adaptation of coral-associated *Endozoicomonas* to their host is evidenced by the gain and loss of several functions that likely increase the fitness inside the host (Fig. 5). Particularly, the ability of *E. acroporae* to synthesize the key steroid hormones testosterone and progesterone from cholesterol confirms that the elevated levels of these molecules at the same temperature in the holobiont may be partially or wholly driven by *Endozoicomonas* symbionts, coinciding with the inability of the coral host to provide these hormones due to exposure to severe heat stress at 34°C. The capacity of bacteria to manipulate eukaryotic steroid hormones has been discovered in human gut microbiota, e.g., *Bacteroides acidifaciens* and *Ruminococcus gnavus*, and is believed to play major roles in controlling steroid hormone stocks in mammals[45]. The discovery of a similar metabolic capacity in coral-associated bacteria suggests bacteria like *Endozoicomonas* can influence major functions in their host.

Steroid hormones play critical roles in many physiological functions, such as growth, reproduction, and development in vertebrates[46]. Their role and origin in invertebrates has been subject to recent debate[47], but there is agreement that steroid hormones do play an important

role in corals, including during gametogenesis and spawning and exogenous additions of steroid hormones influence the coral microbiome composition[48]. Testosterone has been shown to regulate mitochondrial gene expression to counteract the effects of oxidative stress on transcription[49], which in turn increases energy availability for host cells. More importantly, progesterone, testosterone and estradiol-17β function as interkingdom signals between eukaryotic hosts and microbial pathogens[50].

As interkingdom signals, progesterone and testosterone exhibit suppressing effects on pathogenic microbes that may be infecting the coral host during thermal stress, namely bacteria and fungi. For example, testosterone inhibits the growth rates of several pathogens, such as the parasite *Trichomonas vaginalis*, and the bacteria *Enterococcus faecalis*, *Pseudomonas aeruginosa*[51], *Staphylococcus aureus*, *S. epidermidis*, *Bacillus subtilis, B. cereus*, *Listeria monocytogenes* and the fungi *Candida albicans* and *Saccharomyces cerevisiae*, to name a few[7]. Testosterone and progesterone also prime innate immunity by increasing resistance to pathogens, such as *Streptococcus pneumoniae*[52]. Progesterone is also a potent inhibitor of respiration of *Neisseria gonorrhoeae*[53]. Cumulatively, these lines of evidence suggest that testosterone and progesterone may induce coral innate immune responses that are required by the host to respond to potential pathogens. Future research is needed to comprehensively examine coral responses to steroid hormones and their role in coral holobiont homeostasis.

Conclusion

Recent evidence suggests the origin of protosteroids occurred up to 1.6 billion years ago[54], coinciding with the proposed evolution of unicellular eukaryotes. This correlation may indicate an early role for steroids in interkingdom symbiosis that enabled prokaryotes, such as *Endozoicomonas*, to acquire the capacity to convert steroids likely to maintain close associations with animals. The limited distribution of this function across bacterial taxa associating with higher organisms suggests these microbes occupy a unique niche that distinguishes them from other microbiome members. In conclusion, our work proposes a broader role for steroids in the coral holobiont, and highlights the significance of understanding coral symbiosis in efforts to protect endangered coral reef habitats in a changing climate.

Main Figures

Figure 1. Sampling scheme and integrated metagenome/metabolome analysis of the coral holobiont. a, *A. pharaonis* colonies were sampled across a temperature gradient before gDNA and metabolites were extracted for metagenome and metabolome analyses. Samples were collected during thermal stress at 32°C and 34°C (August/October) and recovery at 27°C (June). Additional samples showing white syndrome disease phenotypes at 32°C and 34°C were also collected when found (L: lesion, AL: adjacent to the lesion). **b**, Distribution of metagenomic reads from coral holobiont metagenomes that belong to coral, *Cladocopium spp.* (algal symbiont) and putative microbial members. **c,** Principal component analysis (PCA) of the metabolomics data (n=3491 molecular features). Samples collected at 27°C and 32°C cluster together and are significantly different from samples collected at 34°C (adjusted p-value <0.01), explaining 30.7% of variance. L and AL samples clustered separately from other samples and showed high inter-colony variance (95% confidence area). **d,** Principal coordinate analysis (PCoA) of microbial communities using Bray-Curtis distance shows significant differences between AL and L samples and all other samples (PERMANOVA: R-squared= 0.723; p-value < 0.001). **e,** Distribution of host-symbiont-bacterial genes (left) and metabolites (right) across the top most abundant metabolic pathways. Numbers in parentheses refer to the number of genes and metabolites shown in each pathway, respectively.

Figure 2. Abundance and putative functional importance of *Endozoicomonas acroporae* MAG10. a, Relative abundance of tissue-associated MAGs inferred by read recruitment across all samples. Taxonomic classifications of MAGs are shown in Supplementary Table 4. **b**, *Endozoicomonas* pangenome and BGCs of 28 genomes and MAGs. Pangenomic analyses based on the occurrence of gene clusters were implemented in Anvi'o. The central dendrogram represents hierarchical clustering based on the presence (colored) or absence (opaque) of 37,310 gene clusters. Each track in the pangenome represents a single genome colored according to its isolation source. Genomes were clustered based on their pairwise average nucleotide identity (ANI) values shown in the heatmap, which reveals high similarity of MAG10 to *E. acroprae* genomes. Distribution of BGCs across genomes is shown below the heatmap. Key genes related to aerobactin biosynthesis (*iucC*) and degradation of steroid hormones (*kstD*), galactose (*galM*), and histidine (*hutH*) are labeled on the periphery of the pangenome.

Figure 3. Loss of the aerobactin BGC in symbiotic *Endozoicomonas*. **a**, Genomic neighborhood structure of the aerobactin BGC in genomes of MAG10, *Endozoicomonas* spp. symbiotic with *Acropora* corals, soft corals, their closest free-living isolate, SM1973, and the aerobactin-producer *Klebsiella pneumoniae*. Blue shading across genes represents genes related to aerobactin biosynthesis and uptake. Aerobactin is biosynthesized by 4 genes, one of which is duplicated (*iucA/iucC*). Although the aerobactin biosynthesis operon is partially truncated in the MAG10 scaffold, we confirmed that the only partially truncated gene is the duplicated *iucA/iucC*. The TonB-dependent receptor refers to the aerobactin transporter, *iutA*. **b**, Phylogenetic tree of the aerobactin canonical biosynthesis gene (aerobactin synthase; *iucA/iucC*) shows sequence divergence of *Endozoicomonas* associated with *Acropora* and soft corals from SM1973 and the pathogen *K. pneumoniae*. Circle size indicates bootstrap values. **c**, CAS assay of culture, cells and supernatant of *E. acroporae* AT381 grown in minimal media. EDTA was used as a positive control, while the media was used as a negative control.

Figure 4. Steroid hormone biosynthesis and degradation in the holobiont. a, Steroid biosynthesis and degradation pathways based on coral and MAG10 contigs. Arrows indicate genes and arrow heads correspond to the number of genes carrying a transformation. Metabolite relative abundance is shown for each molecule. Middle: Circos plot depicting all detected steroid-related metabolites and their connection to genes of *Endozoicomonas* (*Endozoic.*), the coral host, or *Cladocopium* spp. (Cl). Colors indicate different pathways (brown=steroid biosynthesis, red=steroid hormone biosynthesis, blue=steroid degradation, black=metabolites). **b,** Proposed cholesterol transformation pathway by *Endozoicomonas* inferred from metabolomics analysis of *E. acroporae* with or without cholesterol. Metabolites with chemical structures denote those with confirmed MS^2 fragmentation; * indicates statistical significance in treatment relative to controls (T-test, p-value <0.05); metabolites with grey names were not detected but inferred based on relevant KEGG pathways. Heatmap shows normalized relative abundance of each metabolite in triplicates in treatment and controls. **c**, MS^2 fragmentation spectra for progesterone (96% match) and testosterone (99.3% match) with measured spectra (top) and reference spectra (bottom). The shown structures indicate the characteristic fragments for each molecule.

Figure 5. Model for Coral-*Endozoicomonas* symbiosis during homeostasis and thermal stress compared to a free-living lifestyle. In a free-living lifestyle, *Endozoicomonas* closest free-living relative synthesizes a large number of secondary metabolites, including for chemical warfare with other microbes and Fe acquisition. *Endozoicomonas* in symbiosis loses many of these BGCs, including loss of Fe-acquisition via aerobactin. At homeostasis, *Endozoicomonas*

with its host by providing amino acids, B vitamins, and is capable of producing glutamate from histidine, degrading Symbiodiniaceae-derived galactose, and utilizing host-derived cholesterol as a carbon source. During thermal stress, *Endozoicomonas* continues to use cholesterol for growth, but also partially transforms it to testosterone and progesterone, which may act in energy availability to the host by reducing oxidative stress, interkingdom signaling, priming innate immunity to increase resistance to pathogenic bacteria and enhancing growth/reproduction.

Data Availability

Metagenomic reads of the coral holobiont community consortium are deposited in NCBI under the BioProject PRJNA1001615. Metagenomically assembled genomes and their annotations are available on Zenodo (https://zenodo.org/deposit/8210635). The untargeted mass spectrometry data is deposited on Global Natural Products Social Molecular Network server (https://gnps.ucsd.edu/) as raw data and can be accessed under this accession number MSV000092488.

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Author contributions

MAO, DA and SAA conceived the project; MAO, DA and TDH conducted the sampling; MAO, TDH and LSYC processed the samples; MAO, ARM and SAA carried out data processing and analysis; MAO, ARM and SAA drafted the manuscript; all authors contributed to manuscript preparation; SAA acquired funding for the project.

Competing Interest Declaration

The authors declare no competing interests.

Additional information

This paper is accompanied by extended data and supplementary information.

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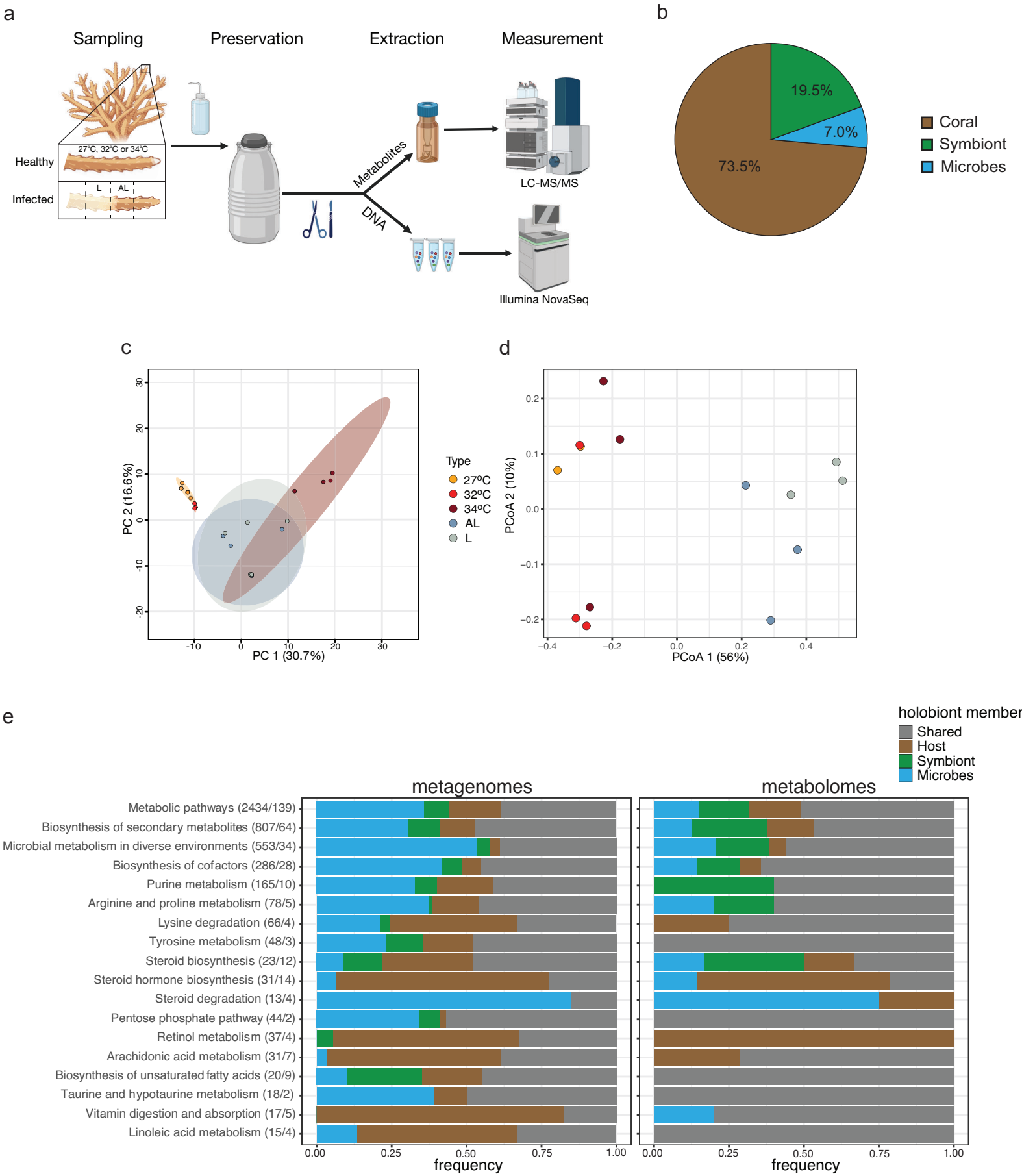
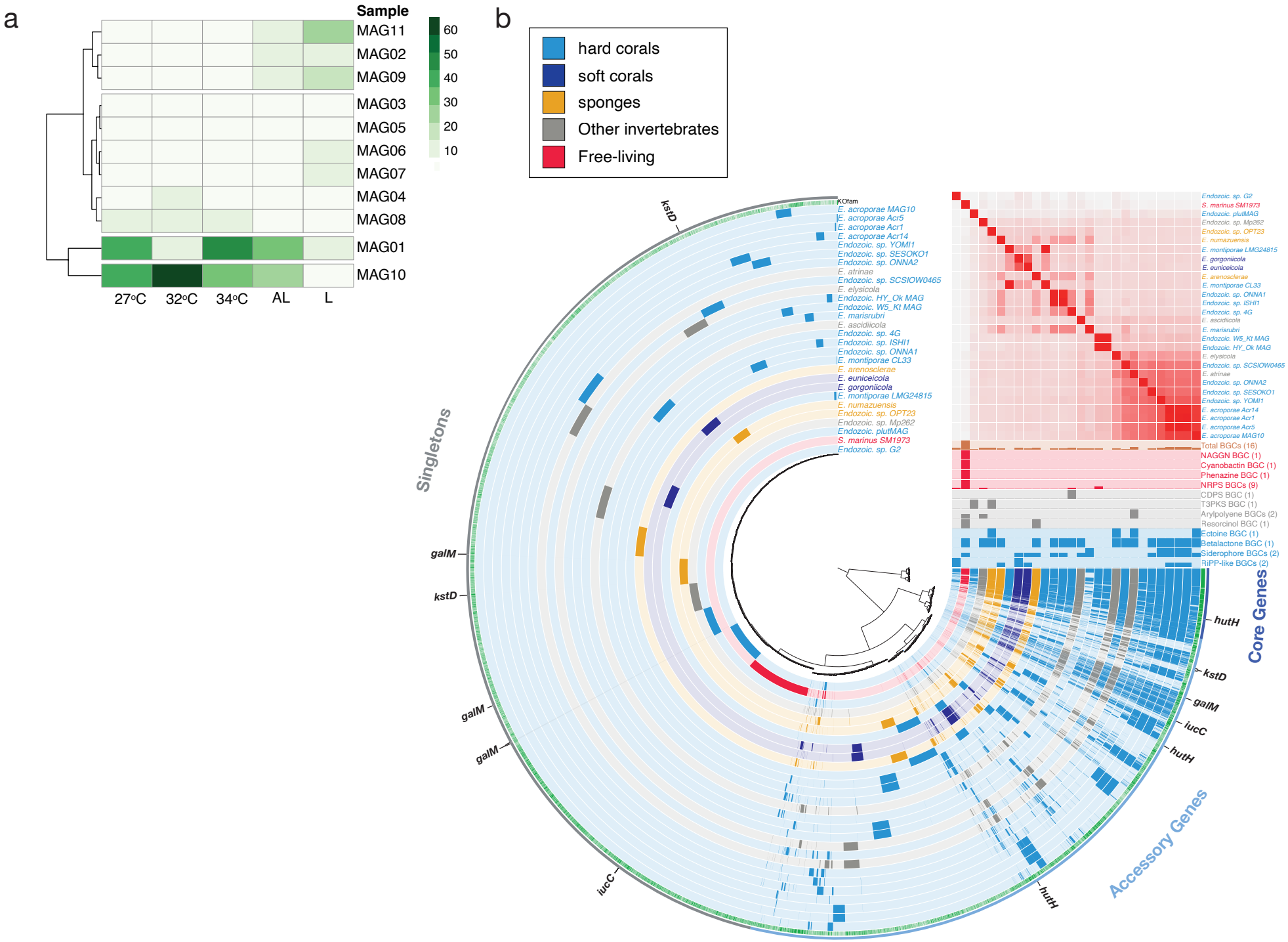
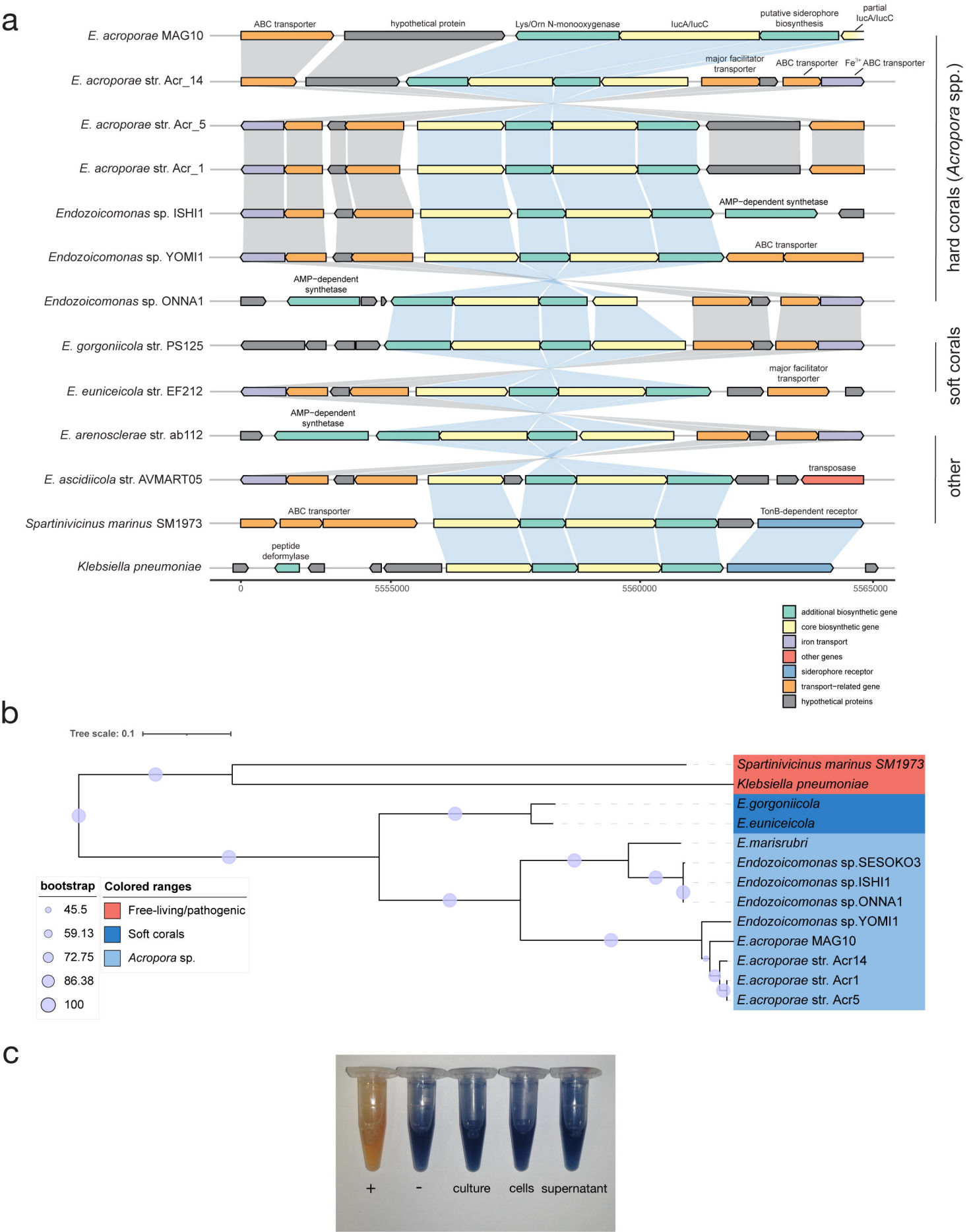
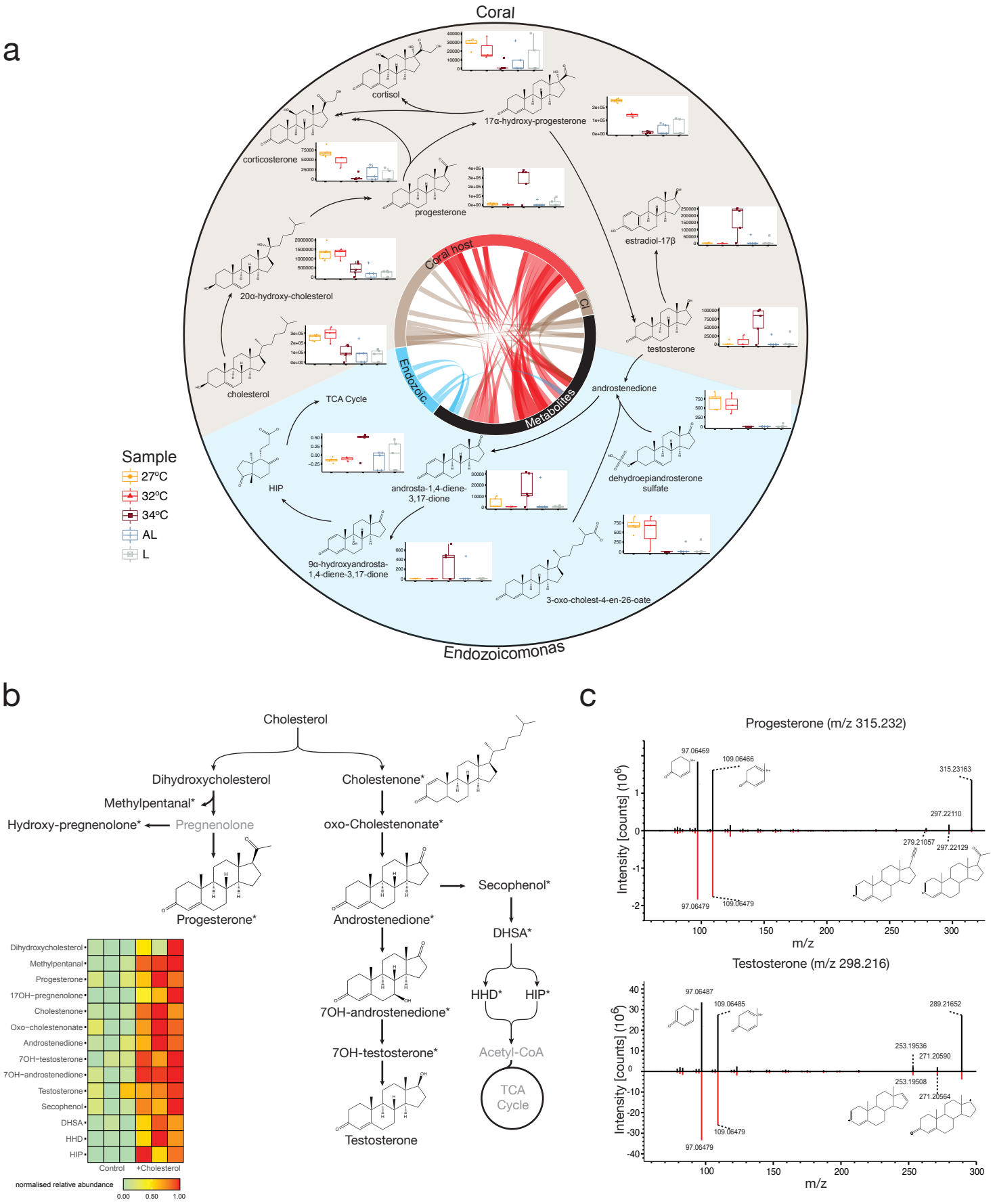
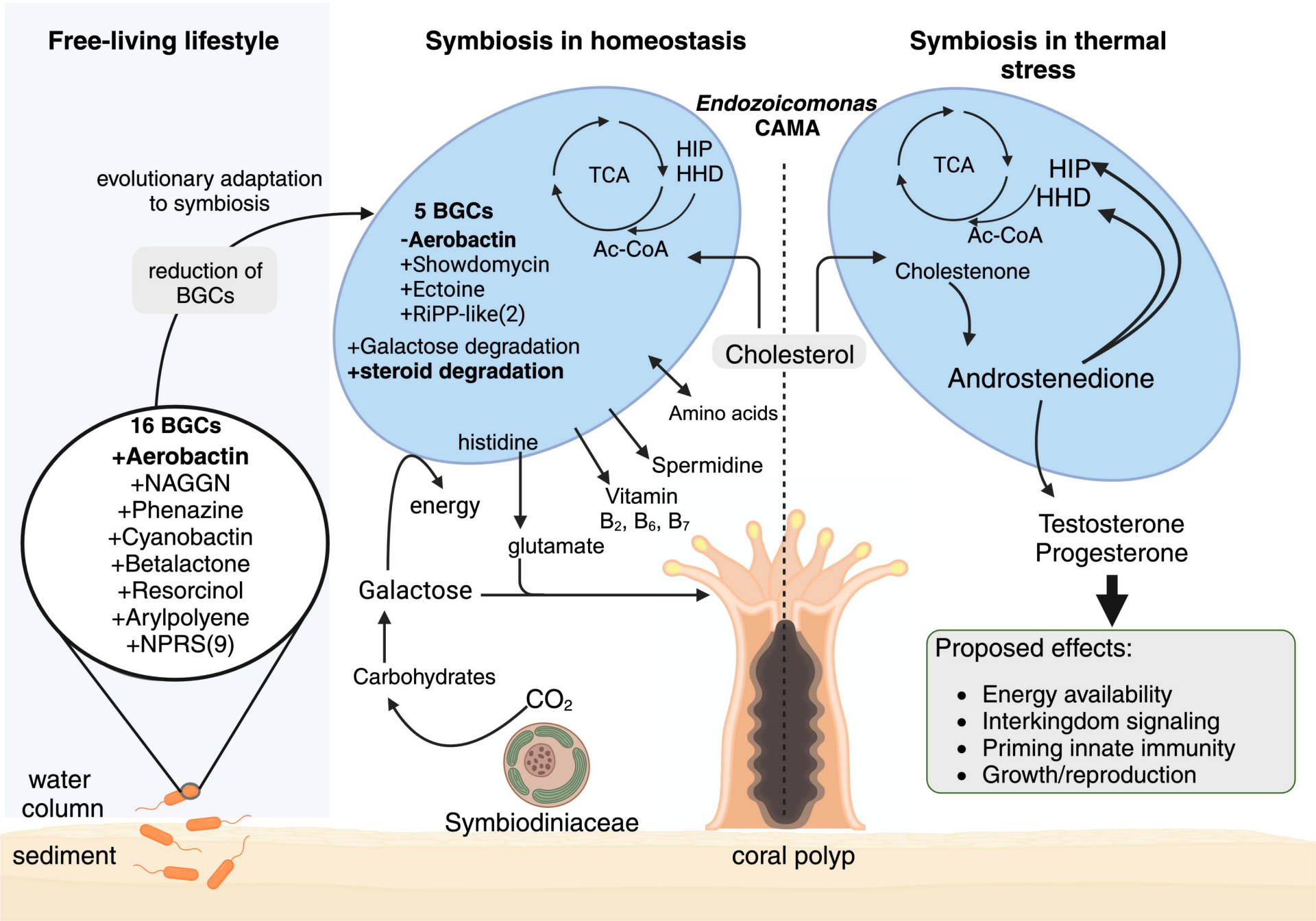

a
Sampling
Preservation
Extraction
Measurement
Healthy
Infected
27°C, 32°C or 34°C
L
AL
Metabolites
DNA
LC-MS/MS
Illumina NovaSeq
b
19.5%
7.0%
73.5%
Coral
Symbiont
Microbes
c
PC 2 (16.6%)
PC 1 (30.7%)
Type
27ºC
32ºC
34ºC
AL
L
d
PCoA 2 (10%)
PCoA 1 (56%)
e
holobiont member
Shared
Host
Symbiont
Microbes
metagenomes
metabolomes
Metabolic pathways (2434/139)
Biosynthesis of secondary metabolites (807/64)
Microbial metabolism in diverse environments (553/34)
Biosynthesis of cofactors (286/28)
Purine metabolism (165/10)
Arginine and proline metabolism (78/5)
Lysine degradation (66/4)
Tyrosine metabolism (48/3)
Steroid biosynthesis (23/12)
Steroid hormone biosynthesis (31/14)
Steroid degradation (13/4)
Pentose phosphate pathway (44/2)
Retinol metabolism (37/4)
Arachidonic acid metabolism (31/7)
Biosynthesis of unsaturated fatty acids (20/9)
Taurine and hypotaurine metabolism (18/2)
Vitamin digestion and absorption (17/5)
Linoleic acid metabolism (15/4)
frequency
frequency


a
Sample
MAG11
MAG02
MAG09
MAG03
MAG05
MAG06
MAG07
MAG04
MAG08
MAG01
MAG10
27°C
32°C
34°C
AL
L
60
50
40
30
20
10
b
hard corals
soft corals
sponges
Other invertebrates
Free-living
KOfam
E. acroporae MAG10
E. acroporae Acr5
E. acroporae Acr1
E. acroporae Acr14
Endozoic. sp. YOMI1
Endozoic. sp. SESOKO1
Endozoic. sp. ONNA2
E. atrinae
Endozoic. sp. SCSIOW0465
E. elysicola
Endozoic. HY_Ok MAG
Endozoic. W5_Kt MAG
E. marisrubri
E. ascidiicola
Endozoic. sp. 4G
Endozoic. sp. ISHI1
Endozoic. sp. ONNA1
E. montiporae CL33
E. arenosclerae
E. euniceicola
E. gorgoniicola
E. montiporae LMG24815
E. numazuensis
Endozoic. sp. OPT23
Endozoic. sp. Mp262
Endozoic. plutMAG
S. marinus SM1973
Endozoic. sp. G2
Total BGCs (16)
NAGGN BGC (1)
Cyanobactin BGC (1)
Phenazine BGC (1)
NRPS BGCs (9)
CDPS BGC (1)
T3PKS BGC (1)
Arylpolyene BGCs (2)
Resorcinol BGC (1)
Ectoine BGC (1)
Betalactone BGC (1)
Siderophore BGCs (2)
RiPP-like BGCs (2)
Singletons
kstD
galM
kstD
galM
galM
iucC
hutH
kstD
galM
iucC
hutH
Core Genes
Accessory Genes


a
E. acroporae MAG10
E. acroporae str. Acr_14
E. acroporae str. Acr_5
E. acroporae str. Acr_1
Endozoicomonas sp. ISHI1
Endozoicomonas sp. YOMI1
Endozoicomonas sp. ONNA1
E. gorgoniicola str. PS125
E. euniceicola str. EF212
E. arenosclerae str. ab112
E. ascidiicola str. AVMART05
Spartinivicinus marinus SM1973
Klebsiella pneumoniae
ABC transporter
hypothetical protein
Lys/Orn N-monooxygenase
IucA/IucC
putative siderophore biosynthesis
partial IucA/IucC
major facilitator transporter
ABC transporter
Fe3+ ABC transporter
AMP−dependent synthetase
ABC transporter
AMP−dependent synthetase
major facilitator transporter
AMP−dependent synthetase
transposase
ABC transporter
TonB-dependent receptor
peptide deformylase
0
5555000
5560000
5565000
hard corals (Acropora spp.)
soft corals
other
additional biosynthetic gene
core biosynthetic gene
iron transport
other genes
siderophore receptor
transport−related gene
hypothetical proteins
b
Tree scale: 0.1
Spartinivicinus marinus SM1973
Klebsiella pneumoniae
E.gorgoniicola
E.euniceicola
E.marisrubri
Endozoicomonas sp.SESOKO3
Endozoicomonas sp.ISHI1
Endozoicomonas sp.ONNA1
Endozoicomonas sp.YOMI1
E.acroporae MAG10
E.acroporae str. Acr14
E.acroporae str. Acr1
E.acroporae str. Acr5
bootstrap
45.5
59.13
72.75
86.38
100
Colored ranges
Free-living/pathogenic
Soft corals
Acropora sp.
c
+
-
culture
cells
supernatant


a
Coral
cortisol
17α-hydroxy-progesterone
corticosterone
progesterone
estradiol-17β
20α-hydroxy-cholesterol
testosterone
cholesterol
androstenedione
TCA Cycle
HIP
androsta-1,4-diene-3,17-dione
dehydroepiandrosterone sulfate
9α-hydroxyandrosta-1,4-diene-3,17-dione
3-oxo-cholest-4-en-26-oate
Coral host
Cl
Endozoic.
Metabolites
Sample
27°C
32°C
34°C
AL
L
Endozoicomonas
b
Cholesterol
Dihydroxycholesterol
Cholestenone*
Methylpentanal*
Hydroxy-pregnenolone*
Pregnenolone
oxo-Cholestenonate*
Progesterone*
Androstenedione*
Secophenol*
DHSA*
7OH-androstenedione*
HHD*
HIP*
7OH-testosterone*
Acetyl-CoA
TCA Cycle
Testosterone
Dihydroxycholesterol
Methylpentanal
Progesterone
17OH−pregnenolone
Cholestenone
Oxo−cholestenonate
Androstenedione
7OH−testosterone
7OH−androstenedione
Testosterone
Secophenol
DHSA
HHD
HIP
Control
+Cholesterol
normalised relative abundance
0.00
0.50
1.00
c
Progesterone (m/z 315.232)
97.06469
109.06466
315.23163
297.22110
279.21057
297.22129
97.06479
109.06479
Intensity [counts] (10^6)
m/z
Testosterone (m/z 298.216)
97.06487
109.06485
289.21652
253.19536
271.20590
253.19508
271.20564
97.06479
109.06479
Intensity [counts] (10^6)
m/z


Free-living lifestyle
Symbiosis in homeostasis
Symbiosis in thermal stress
Endozoicomonas CAMA
evolutionary adaptation to symbiosis
reduction of BGCs
16 BGCs
+Aerobactin
+NAGGN
+Phenazine
+Cyanobactin
+Betalactone
+Resorcinol
+Arylpolyene
+NPRS(9)
5 BGCs
-Aerobactin
+Showdomycin
+Ectoine
+RiPP-like(2)
+Galactose degradation
+steroid degradation
TCA
HIP
HHD
Ac-CoA
Cholesterol
Amino acids
histidine
Spermidine
Vitamin B2, B6, B7
energy
glutamate
Galactose
Carbohydrates
CO2
Symbiodiniaceae
Cholestenone
Androstenedione
Testosterone
Progesterone
Proposed effects:
Energy availability
Interkingdom signaling
Priming innate immunity
Growth/reproduction
water column
sediment
coral polyp