Steroid hormone production by coral-associated *Endozoicomonas* during thermal stress

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Abstract

The coral microbiome is believed to play a critical role in sustaining corals and enabling adaptation to thermal stress. A ubiquitous group of coral-associated bacteria, known as *Endozoicomonas*, are hypothesized to provide essential metabolites to corals, though the roles these bacteria play during thermal stress are largely unknown. We demonstrate that *Endozoicomonas* synthesizes the siderophore aerobactin *in vitro*–a trait potentially suppressed *in hospite*. Integrated metagenomics, metabolomics, and lab validations reveal that *Endozoicomonas* is the only microbe capable of metabolising coral-derived testosterone and converting ubiquitous cholesterol into testosterone and progesterone. These hormones accumulate within the coral holobiont during thermal stress, suggesting active microbial involvement in immune priming and stress mitigation. These findings highlight a previously unrecognized mechanism of microbial contribution to coral resilience. Finally, the capacity of bacteria to synthesize eukaryotic steroids implies deep evolutionary connections between microbial metabolism and the origin of steroid-mediated processes in early eukaryogenesis.

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

Introduction

Corals form intricate associations with a diverse microbiome, collectively referred to as the coral holobiont[1]. 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[5–7], the functional roles of bacteria in the coral holobiont remain largely unexplored[2,8], despite their importance for coral health[3,9,10]. Among the myriad of bacteria associated with corals, the genus *Endozoicomonas* has received considerable attention due to its ubiquitous distribution across coral species and geographic locations[11–13] and a correlation between its abundance and coral health[11,14,15].

While *Endozoicomonas* are sometimes present in the coral mucus[16,17], they are primarily localized within coral tissues as cell-associated microbial aggregates (CAMAs)[18]. Based on genomic predictions, *Endozoicomonas* spp. are hypothesized to play a crucial role in coral holobiont metabolism through the recycling of essential nutrients and biosynthesis of B-vitamins, amino acids and DMSP[11,19–21]. Indeed, when exposed to coral tissue extract, *E. marisrubri* activates vitamins B_1 and B_6 biosynthesis and glycolytic processes[22]. However, despite recent efforts[13,21,23–25], a definitive role for *Endozoicomonas* within the coral holobiont and the precise function underlying its symbiotic association is yet to be identified.

In this study, we combine multiomics data (16S rRNA gene amplicons, shotgun metagenomics, and untargeted metabolomics) to unravel the roles played by *Endozoicomonas* during coral response to extreme thermal conditions in the Arabian Gulf, arguably the hottest marine environment on Earth with sea surface temperature reaching 36°C[26]. Because our aim is to shed light on symbiotic interactions in the coral holobiont, our sampling scheme included healthy coral samples, representing interactions between the coral host and a healthy microbiome, as well as samples infected with white syndrome, representing a dysbiotic state, which served as controls. By integrating metagenomic and metabolomic data, we uncover both shared and niche-specific metabolic pathways among members of the coral holobiont. Given the prominence of *Endozoicomonas* as a key member of the healthy coral microbiome, we leverage pangenomic analysis to delineate conserved and unique functions in *Endozoicomonas* lineages. We then perform additional experimental validations of these functional predictions.

We show that *Endozoicomonas* encodes genes for the biosynthesis and uptake of the iron-binding siderophore aerobactin, and confirm its ability to produce it *in vitro*, likely to access bioavailable iron *ex hospite*. In addition, we show that during thermal stress, two terminal steroid

hormones, testosterone and progesterone, are abundant in corals despite the depletion of most their precursors. Since these precursors are required for synthesis of testosterone and progesterone, their absence suggests a source other than the host, or its algal symbiont is providing testosterone and progesterone. We found that *Endozoicomonas* is the only holobiont member to possess the gene repertoire required for testosterone degradation and, surprisingly, can metabolize cholesterol to produce progesterone and testosterone *in vitro*, a unique capability only present in select bacteria. This ability could potentially mitigate the effects of thermal stress, a distinctive role *Endozoicomonas* carries out among all holobiont members.

Results

We monitored and sampled colonies of the coral *Acropora pharaonis*[27] for 16S rRNA gene amplicons, shotgun metagenomics and untargeted metabolomics from Saadiyat reef on the Arabian Gulf off the coast of Abu Dhabi. Sampling occurred across a temperature gradient starting with early thermal stress showing early signs of white syndrome disease on some colonies at 32°C (June), severe thermal stress and further white syndrome progression at 34°C (August), and recovery at 27°C (October) (Extended Data Table 1). Samples were collected from healthy coral tissues at all three temperatures, and from dysbiotic controls (i.e., white-syndrome lesion sites (L) and healthy appearing tissue adjacent to the lesion (AL) at 34°C) (Fig. 1a). All samples were extracted for metabolites and genomic DNA to profile the coral holobiont metabolome and microbiome composition and functional potential.

Divergent metabolite and bacterial profiles in corals under extreme temperature

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. A total of 5878 putative molecular features were predicted based on *in-silico* mass fragmentation network analysis (SIRIUS) (*see* Methods; Supplementary Table 1). Principal component analysis (PCA) patterns were significant (PERMANOVA; F-value = 7.58; R^2 = 0.62; adj. *p*-value=0.001) 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 (June to October) the holobiont metabolic state during early stress and recovery were similar (Fig. 2a; Extended Data Fig. 1). The metabolomes of the diseased samples (L, AL) displayed inter-colony variations and showed a significant separation from all other samples, suggesting a fundamentally different

chemical composition compared to healthy tissue (Fig. 2a). These findings confirm that acroporid corals in the Arabian Gulf exhibit a notable tolerance to temperatures up to 32°C (which can be lethal to corals elsewhere), while exposure to 34°C poses a critical challenge to their overall fitness, which aligns with previous reports[28].

The bacterial microbiome composition, based on 16S rRNA gene amplicons, revealed that disease samples (L, AL) differed significantly from all other samples (PERMANOVA; F-value = 1.98; R^2 = 0.0318; *adj.* p-value = 0.01), suggesting the bacterial microbiome is resilient and only undergoes a significant shift during disease progression (Fig. 2b). Bacterial ASVs belonging to the family Endozoicomonadaceae dominated the microbiome, with an average relative abundance of 60% in healthy samples. In addition, Endozoicomonadaceae persisted under elevated (32°C) and even extreme temperatures (34°C), which was particularly remarkable as they usually disappear during coral exposure to thermal stress[29] (Fig. 2c; Extended Data Fig. 2a). At lesion sites, the relative abundance of Endozoicomonadaceae constituted only 1.3%, while Rhodobacteraceae and Saprospiraceae contributed 23% and 39% of the microbiome, respectively, indicating the proliferation of opportunistic microbes at the expense of Endozoicomonadaceae during disease onset (Fig. 2c; Extended Data Fig. 2a)[30–32].

Integrated metagenome/metabolome analysis implicates the microbiome in steroid hormone metabolism

The metagenomes of coral holobionts produced a total of 589 gigabases (Gb), averaging 42 Gb per sample (Supplementary Table 3; Extended Data Fig. 3). We leveraged publicly available genomes for *Acropora* and *Cladocopium* to partition the holobiont metagenome before assembling coral and algal contigs (see Methods). A total of 257 million reads (~7% of the holobiont reads) were classified as microbial (i.e., non-coral, non-algal reads; Fig. 2d). These microbial reads were used for downstream read-based and assembly-based metagenomic analyses (Extended Data Fig. 2b and 3; Supplementary Table 3). Taxonomic profiling of putative metagenomic microbial reads revealed a diverse coral microbiome primarily dominated by bacteria (95% of the microbial community (Extended Data Fig. 2b). To explore the potential roles of the microbiome, we assigned all genes and putatively annotated metabolites in our metagenomic and metabolomic datasets to members of the coral holobiont. To do this, metagenomic reads assigned to host, algal symbiont or bacteria were assembled and functionally annotated using the KEGG database to a total of 11,333 KEGG Orthology terms[33] (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 microbes (3,029)

and algal symbionts (620) (Fig. 2e; Supplementary Table 4). Using these KO taxonomic assignments, we mapped the putatively annotated metabolites associated with KEGG compounds (284) (Supplementary Table 2). This analysis indicated 148 of these metabolites were shared between different members of the holobiont, while 43 were assigned uniquely to the host, 42 to the algal symbiont and 51 to other microbes (Fig. 2e, 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 microbes dominate biosynthesis of cofactors, biosynthesis of secondary metabolites, and steroid degradation (Fig. 2e).

To assign functions to specific bacterial taxa, we attempted to assemble metagenomically assembled genomes (MAGs) from our metagenomes. Coral samples devoid of mucus and seawater yielded 11 medium to high-quality MAGs (completeness >75% and contamination <10%). Core microbiome members were defined as MAGs consistently present at relatively high (40-60%) abundance in healthy tissues (at all temperatures) compared to all other MAGs, but showing reduced abundance in dysbiotic samples (AL and L) (Fig. 3a). Out of the 11 MAGs, two MAGs stood out as important members of the coral holobiont: MAG01 assigned to the phylum Myxococcota and MAG10 assigned to the genus *Endozoicomonas* (Extended Data Fig. 4; Supplementary Table 5). *Endozoicomonas* (MAG10), with a 91% completeness and a 1.4% contamination, exhibited a dynamic abundance profile as its relative abundance peaked at 32°C during early stress (65.4%), followed by a dramatic decrease at 34°C (35.8%) and then bounced back with a modest abundance during recovery at 27°C (43%) (Fig. 3a). Consistent with 16S rRNA gene amplicon data, the relative abundance of MAG10 dropped drastically in L samples (0.8%), coinciding with dysbiosis. Given its dynamic abundance pattern and strong association with healthy coral states, we next explored the functional potential of MAG10 to better understand its role within the coral holobiont.

Pangenome analysis reveals conserved functions across *Endozoicomonas* genomes

Since culturing efforts of *Endozoicomonas* from *Acropora* in the Arabian Gulf were not successful, we isolated *Endozoicomonas acroporae* AT381 from *A. kenti* from the Great Barrier Reef (GBR), referred to hereafter as AT381 (Fig. 1b), and obtained a nearly complete genome (size = 6.02 Mbp; completeness = 99.1%) predicted to encode 5001 coding sequences (CDS). AT381 and MAG10 are closely related as they share an average nucleotide identity (ANI) of 94.3% according to OrthoANI[34]. Phylogenomic analysis between MAG10, AT381 and publicly

available *Endozoicomonas* genomes further confirmed the close relationship between MAG10, AT381 and other *E. acroporae* genomes (Extended Data Fig. 5). Functional conservation and specialization across all *Endozoicomonas* genomes/MAGs (MAG10, AT381 and 27 publicly available genomes/MAGs from diverse invertebrate hosts) were examined by constructing a pangenome of their 34,846 gene clusters (Fig. 3b, Supplementary Table 6)[35].

We hypothesized that *Endozoicomonas* genomes symbiotic with corals possess specialized, but shared functions enabling interactions with their coral hosts, compared to genomes isolated from other hosts. As reported for other genomes of coral-associated *Endozoicomonas* spp., MAG10 harbors genes related to symbiosis establishment, e.g., flagellar assembly, type IV pili[18], ankyrin and tetratricopeptide repeats[21,22], several secretion systems involved in host infection (T2SS, T3SS, T6SS)[18,21–23], the genes required for the biosynthesis of the vitamins riboflavin (B_2), biotin (B_7) and pyridoxal-phosphate (B_6), and nine essential amino acids and their transporters (Extended Data Fig. 4bc, Supplementary Table 7)[11]. MAG10 was also significantly enriched within the holobiont in the production of antioxidants[22], heme biosynthesis[25], glycoside hydrolases that cleave the most abundant marine polymers, starch and chitin[36], and diverse ABC transporters (Supplementary Tables 7 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[37], also a common feature in most *Endozoicomonas* genomes, **(c)** biosynthesizing the nucleoside antibiotic showdomycin, **(d)** biosynthesizing and exporting the polyamine spermidine, a reactive oxygen species scavenger, and export proteins for its precursor putrescine, **(e)** and biosynthesizing ectoine, a bacterial osmoprotectant (Fig. 3b, Extended Data Fig. 4c, Extended Data Tables 3 and 4, Supplementary Table 7). Cumulatively, these functions may enhance symbiosis and increase the coral host and/or Symbiodiniaceae fitness, especially during thermal stress.

Endozoicomonas **acquires iron using the siderophore aerobactin only** ***in vitro***

Siderophores are small molecules secreted by some bacteria and fungi to acquire iron in iron-limited environments[38] and can play important roles in iron cycling and bioavailability in the pelagic ocean[39,40]. The genome of *Klebsiella pneumoniae* possesses the canonical, complete gene cluster/operon containing aerobactin synthases (*iucA/iucC*) and the TonB-dependent uptake receptor (*iutA*) used to produce apo-aerobactin and take up Fe-bound aerobactin, respectively[41]. In contrast, MAG10, all other *E. acroporae* and 8 additional coral- and

invertebrate-associated *Endozoicomonas* genomes possessed only the biosynthesis genes within the gene cluster, while having either a slightly distal *iutA* that is outside of the gene cluster in the case of AT381 or a homolog of *iutA* in a completely separate region of the genome for all other *Endozoicomonas* that possess the gene cluster (Fig. 4a, Extended Data Table 4). Aerobactin requires a functional *iutA* for Fe acquisition to function[42] and a distal *iutA* suggests a potential decoupling from biosynthesis. To confirm whether aerobactin utilization by *Endozoicomonas* is active, we grew multiple *Endozoicomonas* strains *in vitro* under Fe-limiting conditions and tested their siderophore production using the chrome azurol S assay (CAS)[43]. The tested strains include two isolates from *Acropora*: *E. acroporae* AT381 and *E. acroporae* Acr14*,* and *E. montiporae* isolated from *Montipora*. *E. montiporae* was used as a negative control as it does not possess aerobactin biosynthesis genes (Extended Data Table 4). The CAS assay showed that *E. acroporae* AT381 and Acr14 were able to grow and utilize Fe from CAS (Fig. 4b) while *E. montiporae* was not able to grow, consistent with its inability to produce aerobactin. These findings suggest that select *Endozoicomonas* strains and species (12 out of 27 in total) can utilize aerobactin for Fe acquisition *in vitro* and likely *ex hospite*. We were not able to detect any m/z signatures that match aerobactin, its Fe-complex or photoproducts[44] in the holobiont metabolomes. Indeed, siderophore production *in hospite* is mainly associated with pathogenic bacteria[45], and siderophore production may take place when *Endozoicomonas* are in seawater *ex hospite*.

The fact that every examined *Endozoicomonas* genome capable of biosynthesizing aerobactin possesses distal, not proximal, *iutA* homologs indicates a potential decoupling of expression between biosynthesis and transport, which can make Fe acquisition less efficient and more energetically costly for the bacteria. This pattern also suggests a gradual loss of function of the transporter that would eventually abolish aerobactin biosynthesis over evolutionary timescale. Indeed, a loss of function was concluded from the lack of *iutA* in the genomes of *Bacillus licheniformis* and *B. halodurans* despite the presence of the aerobactin biosynthesis operon[46].

To assess the evolutionary pressures acting on the *iutA* gene, we calculated rates of non-synonymous and synonymous substitutions (dN/dS) ratios across multiple *E. acroporae* strains compared to the housekeeping gene DNA Polymerase III subunit delta as a conserved core gene against *E. acroporae* AT381 as a reference (Supplementary Table 9). Several *Endozoicomonas* isolates from *Acropora* displayed signatures of relaxed purifying selection (significantly elevated dN/dS) in *iutA*, suggesting ecological redundancy or diminished reliance on siderophore-mediated iron acquisition in certain niches. In contrast, *iutA* in *E. acroporae* Acr14 exhibited low dN/dS value (0.1), consistent with strong purifying selection and functional

conservation. These patterns suggest that while some *Endozoicomonas* strains may reduce dependence on siderophore systems, others retain this capability, potentially as a survival strategy during transitions to a free-living lifestyle *ex hospite*. MAG10 was not included in this analysis because metagenome assembled genomes are typically a mosaic of sequences from multiple closely related strains, rather than a single, clonal genome. This chimerism, along with assembly artifacts, can distort substitution rate estimates and obscure true evolutionary signals[47]. Thus, we propose that coral-associated *Endozoicomonas* do not utilize the uptake of siderophores *in hospite*, a function that could be used in the free-living stage, and that over evolutionary time aerobactin-dependent Fe acquisition will be lost. Coral-associated *Endozoicomonas* can instead utilize other pathways to acquire iron, such as heme transporters (Supplementary Table 7). Further work is needed to confirm this hypothesis.

Steroid metabolism is a prominent function of *Endozoicomonas* among the coral microbiome

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*) gene (Fig. 5; Supplementary Table 7). This function was shared with most *Endozoicomonas* genomes (Fig. 3b; Extended Data Table 4), consistent with their broader capacity to breakdown this important steroid molecule[48]. The relative abundance of most steroids gradually and significantly decreased as temperature increased, with abundance at 34°C, AL, and L near background levels (Fig. 5, Extended Data Fig. 6, Supplementary Table 9), consistent with previous reports[49,50] showing suppression of steroid hormones under stress and during infection. Surprisingly, a notable exception to this pattern were select key terminal steroids and steroid degradation products. Specifically, the eukaryotic hormones, progesterone, testosterone and estradiol-17β, in addition to *Endozoicomonas*-derived androsta-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. 5, Supplementary Table 10). Since the sampling timepoints fell outside the spawning season in the Arabian Gulf (March to May)[51], and most precursors of progesterone and testosterone showed low relative abundance near background levels at 34°C, the elevated levels of these steroid hormones during severe stress at 34°C were unexpected. Furthermore, MAG10 is the only microbiome member that possesses a 17-β-hydroxysteroid dehydrogenase (K05296; E1.1.1.51), which has been shown to synthesize testosterone from androstenedione, the reverse reaction of KstD (Supplementary Table 7)[52], we hypothesized that *Endozoicomonas* may provide corals with some of these steroids during severe stress.

MAG10 encodes genes associated with cholesterol binding, such as those containing the steroidogenic acute regulatory protein-related lipid transfer (START) and sterol carrier protein-2 (SCP2) domains, as well as cholesterol/phospholipid transport, including MlaD, MlaE, and ABC transporters (Supplementary Table 7). Given that cholesterol is the precursor of steroid hormones and can be one of the abundant steroid molecules in the coral holobiont[48,53], we investigated whether *Endozoicomonas* possessed the capacity to produce the terminal steroids progesterone, testosterone, or estradiol-17β from cholesterol. Cultures of three *Endozoicomonas* strains, *E. acroporae* AT381, *E. acroporae* Acr14 and *E. montiporae* were grown at 26°C and 34°C in minimal media with or without cholesterol. In addition, these cultures were also grown at the same temperatures and conditions with or without testosterone to confirm its capacity to break it down (Fig. 1b). After 24 hours, all three strains utilized both substrates and accumulated significant amounts of several steroid hormones (based on LC-HRMS and fragmentation information; Fig. 6a,c; Supplementary Table 11) as well as degradation products of testosterone (Fig. 6b), similar to observed results in holobiont samples (Fig. 5; Supplementary Table 10). Specifically, *E. acroporae* AT381 and *E. monitporae* converted testosterone to androstenedione and other products at both temperatures, leading to steroid degradation, which ultimately feeds into the TCA cycle (Fig. 6b). In contrast *E. acroporae* Acr14 carried out similar transformations but only at 34°C, suggesting potential regulation of these transformations at higher temperatures. Surprisingly, *E. acroporae* AT381 converted cholesterol into the terminal products progesterone, testosterone, androstenedione and other intermediates at both temperatures (Fig. 6a, Extended Data Fig. 6c). In contrast, *E. acroporae* Acr14 converted cholesterol to progesterone and cholesterol derivatives only at 34°C, while *E. montiporae* converted cholesterol to either testosterone, androstenedione and related metabolites at 26°C, or to progesterone and cholesterol derivatives at 34°C, indicating a strong temperature influence on the regulation of these transformations (Fig. 6a). These findings suggest that depending on the species/strain of *Endozoicomonas*, a bifurcation of cholesterol conversion towards testosterone or progesterone is controlled by temperature, which may explain the high relative abundance of progesterone and testosterone in the holobiont at 34°C (Fig. 5 and Extended Data Fig. 6a,b).

Using high-resolution mass spectrometry and fragmentation information, we propose a metabolic pathway that bifurcates cholesterol into **(a)** progesterone via pregnenolone and **(b)** testosterone via oxo-cholestenoate and androstenedione (Fig. 6c,d). Although the pangenome and auto-annotation highlight important enzymes for steroid degradation (e.g. *kstD*), further mining of the *E. montiporae* genome yielded steroid delta isomerases of the CyP450 family,

which putatively converts pregnenolone into progesterone (Fig. 6d; Supplementary Table 7)[48]. MAG10 also encodes a member of the 3-beta hydroxysteroid dehydrogenase/isomerase protein family [PF01073.22] (Supplementary Table 7). Orthologs of this protein are present in all three *Endozoicomonas* strains we used for steroid incubations. This enzyme family includes 3β-hydroxysteroid dehydrogenase/Δ□-Δ□ isomerases (3β-HSDs), which plays a central role in steroid biosynthesis by catalyzing the oxidation and isomerization of steroid precursors into active steroid hormones. Although mining *Endozoicomonas* genomes did not reveal any specific exporters for testosterone or progesterone, both hormones are lipophilic and can diffuse freely through the plasma membrane[54–56]. Collectively, these findings suggest *Endozoicomonas* possesses the genetic repertoire not only for steroid degradation, but for their biosynthesis too, positioning them as active contributors to steroid metabolism in the coral holobiont.

Discussion

The cumulative adaptation of coral-associated *Endozoicomonas* to their host is evident by the gain and loss of several functions that are likely to increase their fitness inside the host. 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 in the holobiont when seawater temperature is high may be partially or wholly driven by *Endozoicomonas* symbionts. While many alpha- and gamma-proteobacteria have evolved mechanisms to degrade steroid hormones such as testosterone[48,57,58], the capacity of bacteria to synthesize eukaryotic steroid hormones is not common. The most prominent bacteria synthesizing steroid hormones are specific human gut microbiota, such as *Bacteroides acidifaciens* and *Ruminococcus gnavus*, which are believed to play major roles in controlling steroid hormone stocks in mammals[59]. Moreover, *Mycobacterium smegmatis* was shown to synthesize testosterone and estrogens *in vitro* from steroid precursors[60]. 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[48]. Their role and origin in invertebrates have been subject to recent debate[61]. While definitive CYP19A1 and HSD17B3 orthologs (the vertebrate enzymes that catalyze the final steps in estrogen and androgen synthesis, respectively) have not been identified in invertebrate genomes, several studies have demonstrated functional

aromatase activity in corals (e.g., *Euphyllia ancora*, *Pocillopora damicornis*) using substrate conversion assays and inhibition by vertebrate aromatase inhibitors[62,63] that suggest invertebrates are capable of synthesizing these molecules. We also identified several coral genes involved in steroid hormone biosynthesis, including 17β hydroxysteroid dehydrogenase and several members of the cytochrome P450 enzyme family that can biosynthesize testosterone and progesterone, respectively, as reported in previous coral genomes[64]. This suggests that although the exact gene homologs may differ, the enzyme function is present. However, 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[61,65,66]. Testosterone regulates mitochondrial gene expression to counteract the effects of oxidative stress on transcription[67], 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[68].

As interkingdom signals, progesterone and testosterone can 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 of several pathogens, such as the parasite *Trichomonas vaginalis*, and the bacteria *Enterococcus faecalis*, *Pseudomonas aeruginosa*[69], *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 bacterial pathogens[70–73]. Progesterone is also a potent inhibitor of respiration of *Neisseria gonorrhoeae*[74] and has anti-inflammatory and immunomodulatory properties that can help suppress the overactive immune response during infections, thus reducing tissue damage from uncontrolled inflammation[75,76]. 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[77], coinciding with the proposed evolution of unicellular eukaryotes. This correlation indicates that the origin of these molecules is most likely eukaryotic, especially given their role in sexual reproduction, which is absent in prokaryotes. Through interkingdom interactions, some bacteria

might have inherited select genes from steroid hormone biosynthesis that enabled them to hijack their host immune system and thus pave the way for establishing symbiotic interactions. 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. Because of this selectivity, it may be that bacteria, such as *Endozoicomonas* that can synthesize steroid hormones, act as keystone species that enable other bacteria in the microbiome to also associate with the host. 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.

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/records/15276884). The untargeted mass spectrometry data is deposited on the Global Natural Products Social Molecular Network server (https://gnps.ucsd.edu/) as raw data and can be accessed under the accession numbers MSV000092488 and MSV000097641. Raw 16S rRNA gene amplicon libraries are deposited under the BioProject PRJNA1262123 in NCBI. Raw sequences and reference genome for *E. acroporae* AT381 are deposited under the accession number PRJNA1267713.

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Author contributions

MAO, DA and SAA conceived the project; MAO and DA conducted the sampling; MAO, TDH and LSYC processed the samples; TDH, SA, MAO, and JBR conducted lab experiments; AM, MAO, GMR carried out data processing and analysis; AM, MAO and SAA drafted the

manuscript; AM and MAO plotted all the figures; 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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Figure 1. Sampling, analysis, and validation experiments schemes. **a**, *Acropora pharaonis* colonies were sampled across a temperature gradient before gDNA and metabolites were extracted for microbiome and metabolome analyses. Samples were collected during thermal stress at 32°C and 34°C (June/August) and recovery at 27°C (October). Additional samples showing white syndrome disease phenotypes at 34°C were also collected (L: lesion, AL: adjacent to the lesion). Samples were processed for 16S rRNA gene amplicon, shotgun metagenome, and untargeted metabolome analyses. **b**, Validation experiments were conducted on three different coral-associated *Endozoicomonas* to examine siderophore-based iron acquisition and steroid production.

Figure 2. Integrated microbiome/metabolome analysis of the coral holobiont. a, Principal component analysis (PCA) of the metabolomics data (n=5878 putative molecular features). Samples collected at 27°C and 32°C cluster together and are significantly different from samples collected at 34°C. **b**, Principal coordinate analysis (PCoA) of microbial communities using Bray-Curtis distance shows significant differences between AL and L samples and all other samples. **c**, ASV-based bacterial community structure across temperatures and disease samples at the family level. Each bar plot represents the bacterial abundance of a coral colony. The top 20 bacterial families are shown. **d**, Distribution of metagenomic reads from coral holobiont metagenomes across coral, *Cladocopium spp*. (algal symbiont) and putative microbial members. **e**, Distribution of host-algal symbiont-bacterial genes (left) and metabolites (right) across the top-most abundant KEGG metabolic pathways. Numbers in parentheses refer to the number of genes and metabolites shown in each pathway, respectively.

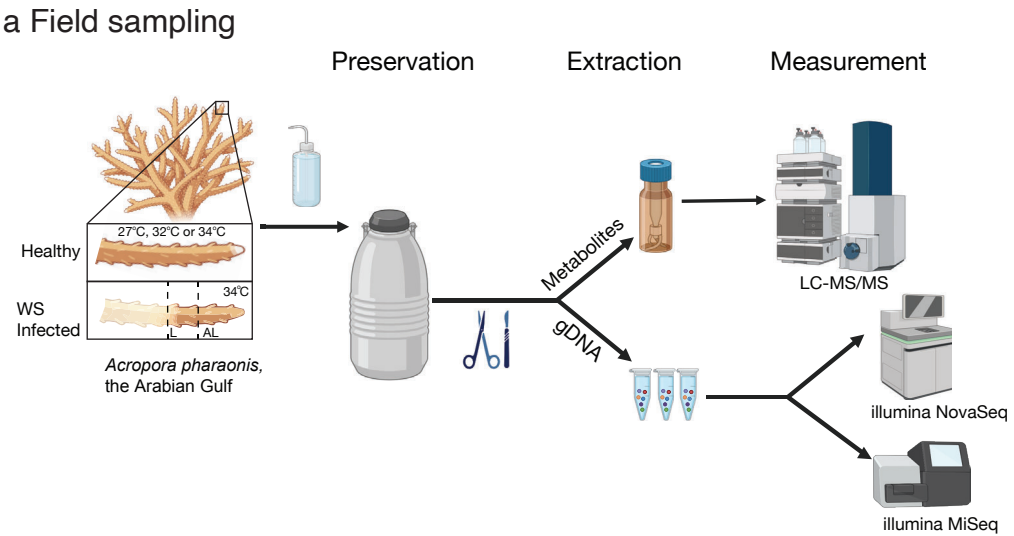
Figure 3. Abundance of *Endozoicomonas* MAG10 and the putative functional importance of *Endozoicomonas*. a, Relative abundance of tissue-associated MAGs inferred by read recruitment from metagenomes across all samples. Taxonomic classifications of MAGs are shown in SI Table 4. **b**, *Endozoicomonas* pangenome and biosynthetic gene clusters (BGCs) of 29 genomes and MAGs, including MAG10 and *E. acroporae* AT381. *Spartinivicinus marina* SM1973 was included to represent a free-living member of the Oceanospirillales as a close free-living isolate to *Endozoicomonas.* Pangenomic analyses based on the occurrence of gene clusters were implemented in Anvi'o[34]. The central dendrogram represents hierarchical clustering based on the presence (colored) or absence (opaque) of 34,846 gene clusters. Branches were colored based on core, accessory or singleton/doubleton genes. 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. acroporae* 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 4. Utilization potential of the siderophore aerobactin for iron acquisition in *Endozoicomonas*. a, Genomic neighborhood organization of the aerobactin BGC in genomes of MAG10, *Endozoicomonas* spp. symbiotic with *Acropora* corals, soft corals, their closest free-living isolate, *S. marinus* SM1973, and the aerobactin-producer *Klebsiella pneumoniae*. Shading

across genes represents gene synteny of aerobactin biosynthesis (blue), aerobactin uptake (orange), or other genes (grey). 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 gene, *iutA,* which is proximal to the BGC of SM1973 and K. *pneumoniae.* Among all coral-associated *Endozoicomonas,* only *E. acroporae* AT381 has *iutA* ~11kb distal to the BGC; all other *Endozoicomonas* genomes display a distal ortholog/paralog of *iutA* elsewhere in the genome (>11kb away from the biosynthesis BGC) **b**, CAS assay of cultures of two *E. acroporae* strains (AT381 and Acr14), encoding the aerobactin cluster, grown in iron-limited media on agar. Siderophore activity was then expressed as forming halos around resultant bacterial colonies. * indicates isolation from the hard coral *Acropora.*

Figure 5. Putative steroid hormone biosynthesis and degradation in the holobiont across the temperature gradient. Steroid biosynthesis and degradation pathways based on coral and Endozoicomonas MAG10 contigs. Arrows indicate genes, and arrowheads correspond to the number of genes carrying a transformation. The relative abundance of putative steroid hormones is presented along with adjusted p-values, calculated using the Benjamini-Hochberg method, based on ANOVA results that assess the impact of temperature on changes in their abundance. Middle: Circos plot depicting all detected steroid-related metabolites and their connection to genes of *Endozoicomonas* (Endozoic.), the coral host, or *Cladocopium* spp. (Cl). The plot shows that steroid degradation is restricted to *Endozoicomonas*, steroid biosynthesis is mostly dominated by *Cladocopium* and steroid hormone biosynthesis is mostly dominated by the coral. Colors indicate different pathways (brown=steroid biosynthesis, red=steroid hormone biosynthesis, blue=steroid degradation, black=metabolites).

Figure 6. Steroid hormone production pathway by *Endozoicomonas*. Two *E. acroporae* (AT381 and Acr 14) and one *E. montiporae* strains were incubated with or without cholesterol (**a**) or testosterone (**b**) at 26°C and 34°C. Heatmaps show the normalized mean relative abundance of each metabolite in each condition, with * indicating statistical significance in treatment relative to controls (T-test, adjusted p-value <0.05). Statistical significance was not calculated for Testosterone in (**b**) because it was added to the media. All detected metabolites were annotated based on the use of accurate mass within 5 ppm error and high-resolution MS/MS fragmentation of standards; additionally, a testosterone authentic standard was used to further confirm its production with the aforementioned criteria as well as retention time. **c,** Novel pathway for the production of progesterone and testosterone inferred from metabolomics analysis. Dotted lines imply the lack of genes involved in the metabolic reactions in the *Endozoicomonas* genomes. **d,** MS^2 fragmentation spectra for progesterone and testosterone with measured spectra (black) and reference spectra (red). The structures shown indicate the characteristic fragments for each molecule.


a Field sampling
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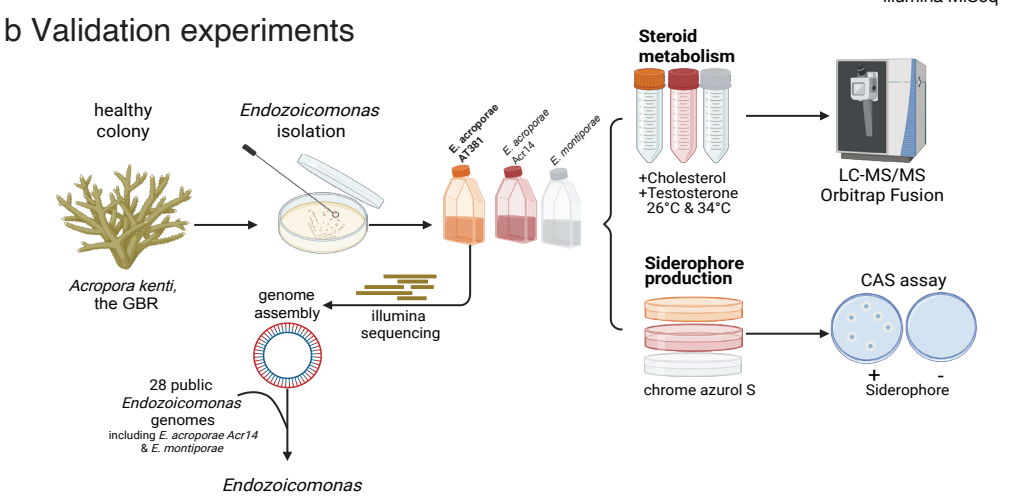
b Validation experiments
healthy
colony
Endozoicomonas
isolation
E. acroporae
AT381
E. acroporae
Acr14
E. montiporae
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the GBR
genome
assembly
illumina
sequencing
28 public
Endozoicomonas
genomes
including E. acroporae Acr14
& E. montiporae
Endozoicomonas
pangenome
Steroid
metabolism
+Cholesterol
+Testosterone
26°C & 34°C
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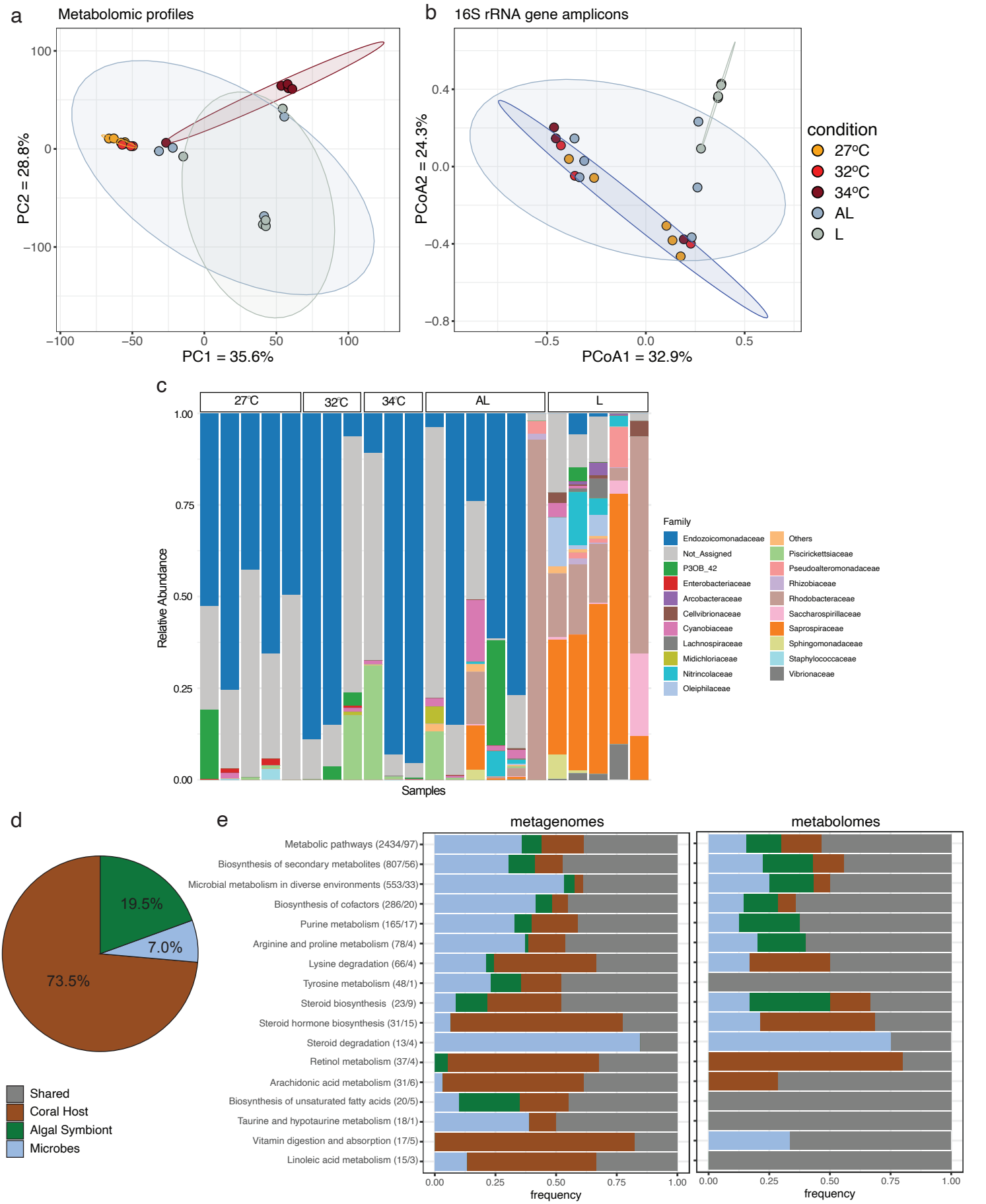
a
Metabolomic profiles
PC2 = 28.8%
PC1 = 35.6%
b
16S rRNA gene amplicons
PCoA2 = 24.3%
PCoA1 = 32.9%
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27ºC
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Relative Abundance
Samples
Family
Endozoicomonadaceae
Not_Assigned
P3OB_42
Enterobacteriaceae
Arcobacteraceae
Cellvibrionaceae
Cyanobiaceae
Lachnospiraceae
Midichloriaceae
Nitrincolaceae
Oleiphilaceae
Others
Piscirickettsiaceae
Pseudoalteromonadaceae
Rhizobiaceae
Rhodobacteraceae
Saccharospirillaceae
Saprospiraceae
Sphingomonadaceae
Staphylococcaceae
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d
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Shared
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e
metagenomes
metabolomes
Metabolic pathways (2434/97)
Biosynthesis of secondary metabolites (807/56)
Microbial metabolism in diverse environments (553/33)
Biosynthesis of cofactors (286/20)
Purine metabolism (165/17)
Arginine and proline metabolism (78/4)
Lysine degradation (66/4)
Tyrosine metabolism (48/1)
Steroid biosynthesis (23/9)
Steroid hormone biosynthesis (31/15)
Steroid degradation (13/4)
Retinol metabolism (37/4)
Arachidonic acid metabolism (31/6)
Biosynthesis of unsaturated fatty acids (20/5)
Taurine and hypotaurine metabolism (18/1)
Vitamin digestion and absorption (17/5)
Linoleic acid metabolism (15/3)
frequency
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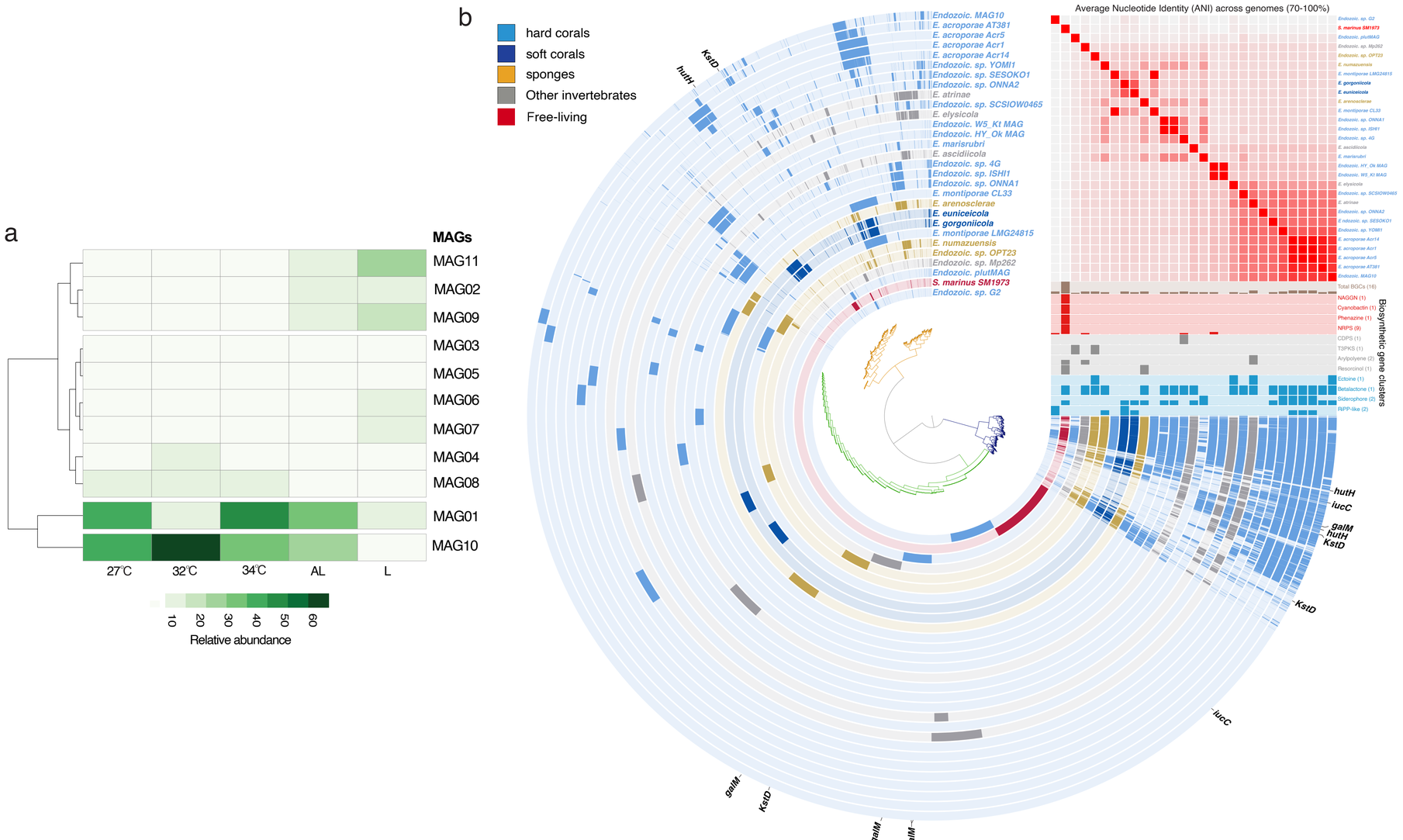
a
MAGs
MAG11
MAG02
MAG09
MAG03
MAG05
MAG06
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MAG08
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MAG10
27°C
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34°C
AL
L
10
20
30
40
50
60
Relative abundance
b
hard corals
soft corals
sponges
Other invertebrates
Free-living
Endozoic. MAG10
E. acroporae AT381
E. acroporae Acr5
E. acroporae Acr1
E. acroporae Acr14
Endozoic. sp. YOMI1
Endozoic. sp. SESOKO1
Endozoic. sp. ONNA2
E. atrinae
Endozoic. sp. SCSIOW0465
E. alysicola
Endozoic. W5_Kt MAG
Endozoic. HY_Ok 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
Average Nucleotide Identity (ANI) across genomes (70-100%)
Total BGCs (16)
NAGGN (1)
Cyanobactin (1)
Phenazine (1)
NRPS (9)
CDPS (1)
T3PKS (1)
Arylpolyene (2)
Resorcinol (1)
Ectoine (1)
Betalactone (1)
Siderophore (2)
RiPP-like (2)
Biosynthetic gene clusters
hutH
KstD
iucC
galM

a

Hard corals (*Acropora* spp.)

others

Endozoicomonas MAG10

ABC transporter

hypothetical protein

Lys/Orn N−monooxygenase

IucA/IucC

putative siderophore biosynthesis

partial IucA/IucC

E. acroporae str. Acr14

E. acroporae str. Acr5

E. acroporae str. Acr1

Endozoicomonas sp. ISHI1

AMP−dependent synthetase

Endozoicomonas sp. ONNA1

Endozoicomonas sp. YOMI1

E. euniceicola str. EF212

E. ascidiicola str. AVMART05

transposase

E. gorgoniicola str. PS125

hypothetical protein

E. arenosclerae str. ab112

AMP−dependent synthetase

*E. acroporae str. AT381**

iutA TonB−dependent receptor

Spartinivicinus marinus SM1973

ABC transporters

Klebsiella pneumoniae

peptide deformylase

5550000

5555000

5560000

5565000

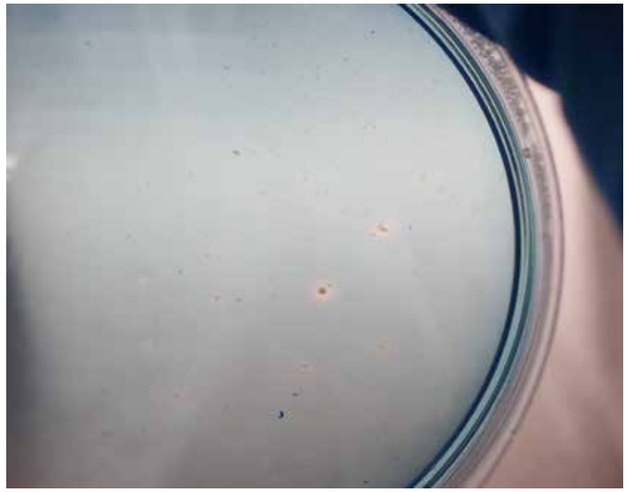
fill

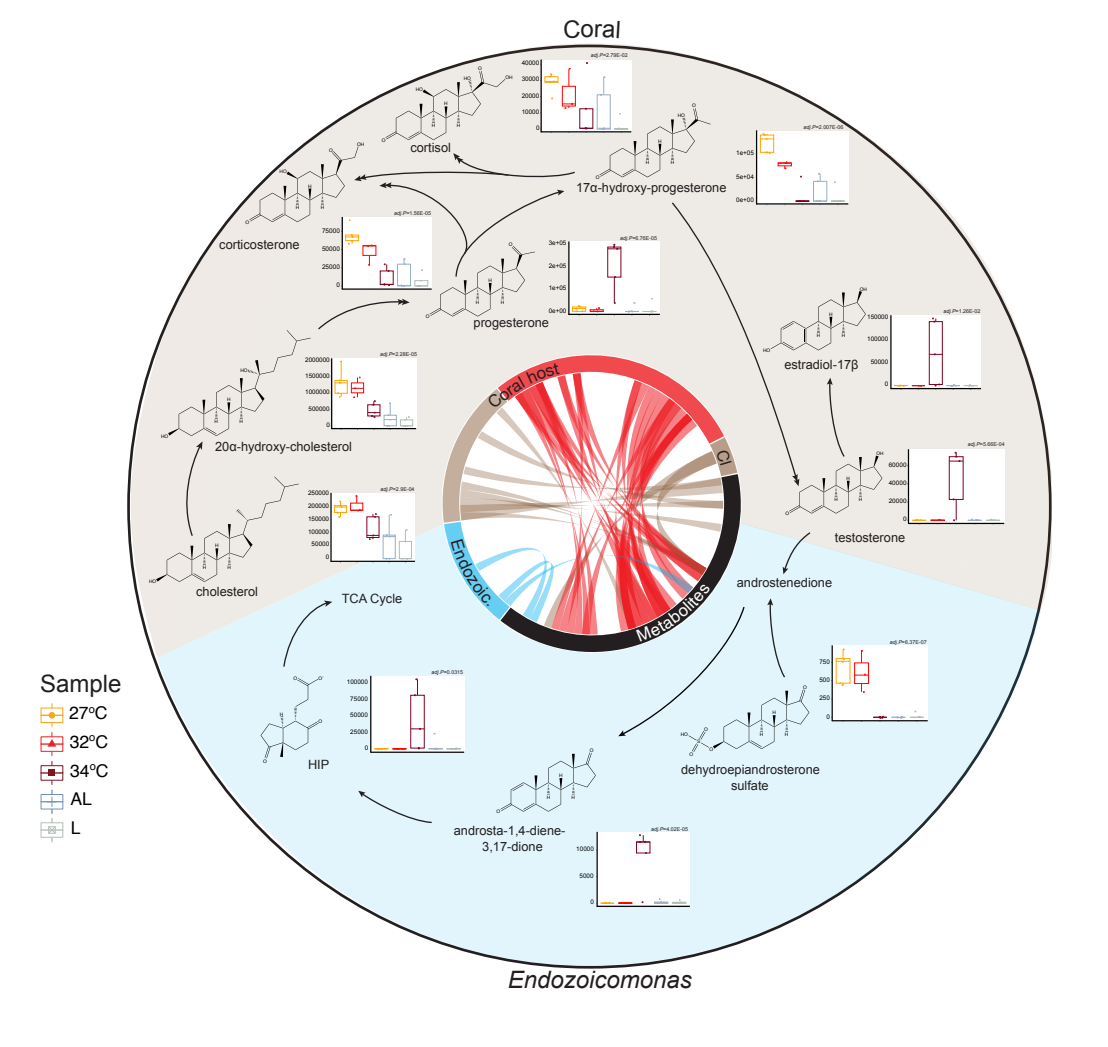
- additional biosynthetic gene
- core biosynthetic gene
- hypothetical protein
- iron transport
- other genes
- siderophore receptor
- transport−related gene

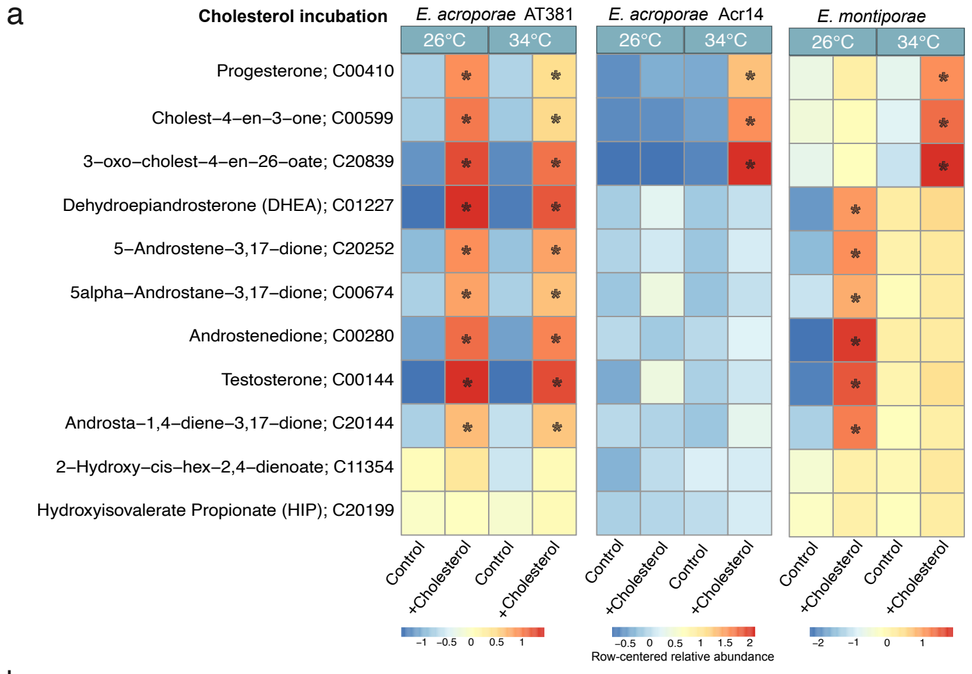
b

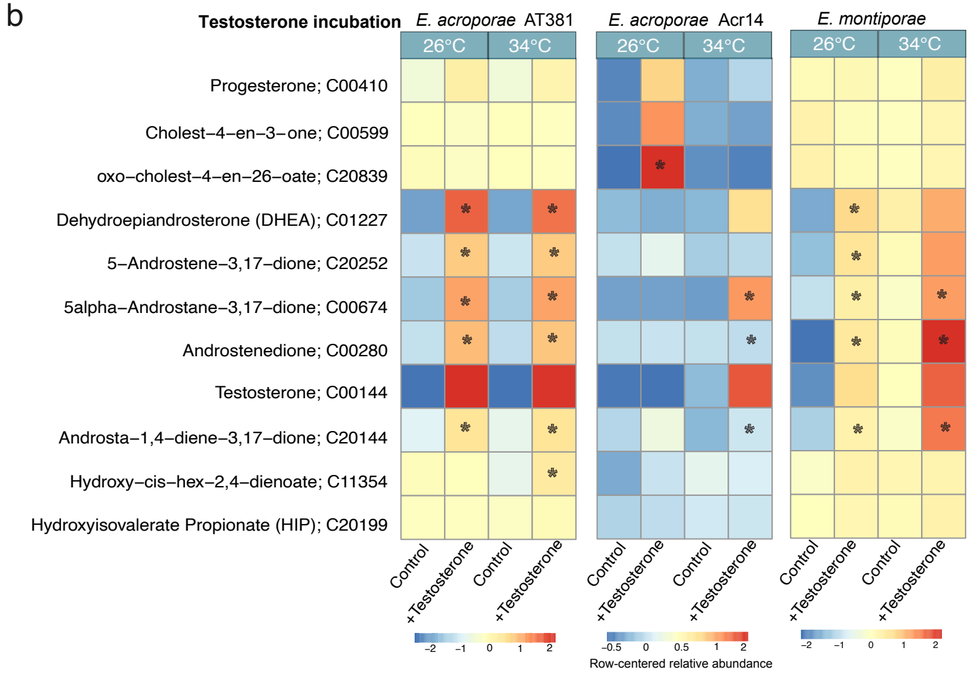
***E. acroporae str.* AT381**

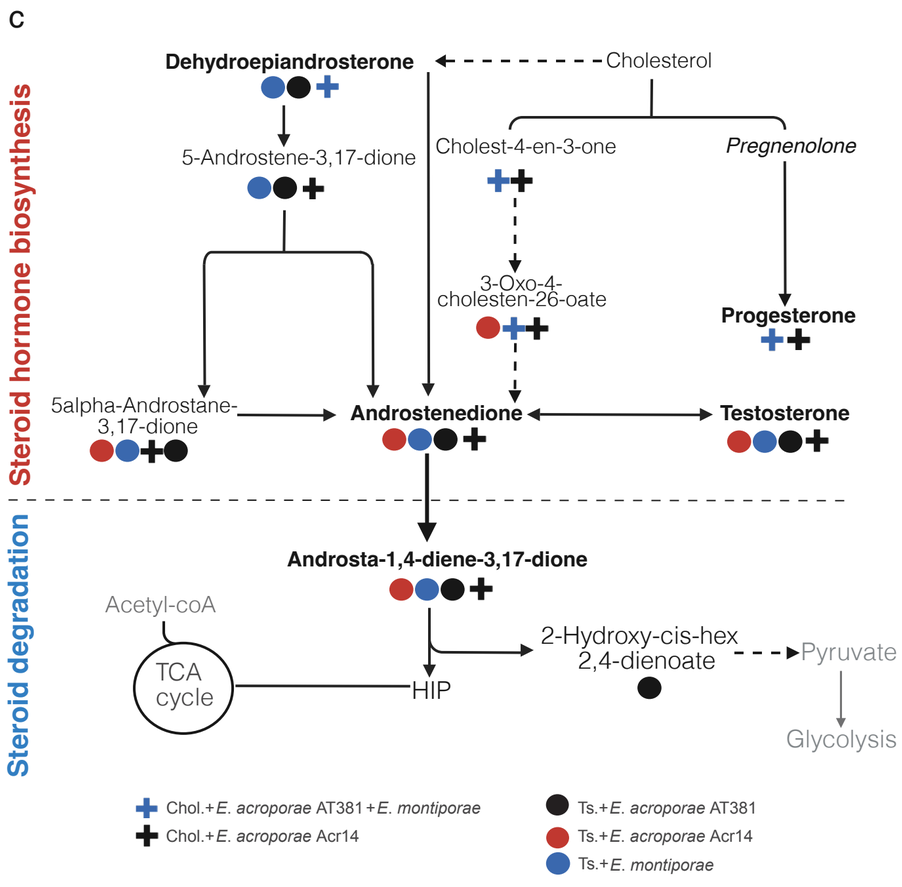
***E. acroporae str.* Acr14**

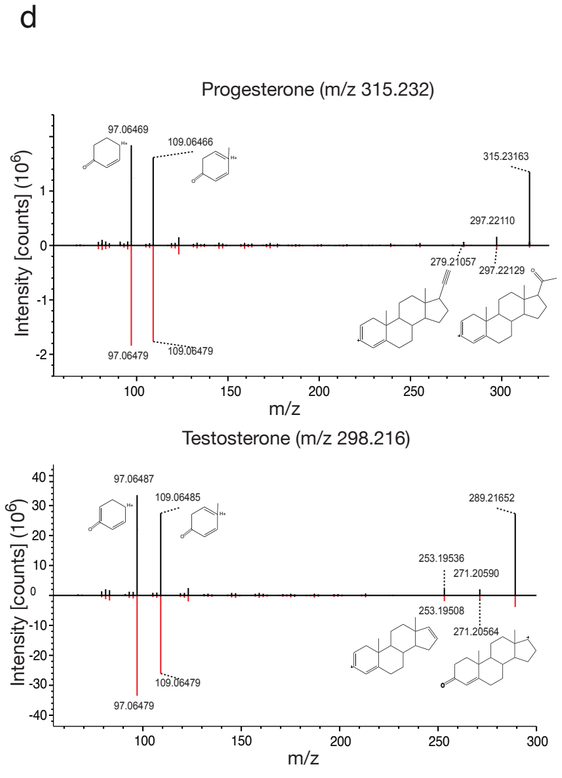



Coral
cortisol
17α-hydroxy-progesterone
corticosterone
progesterone
estradiol-17β
20α-hydroxy-cholesterol
testosterone
cholesterol
androstenedione
TCA Cycle
HIP
dehydroepiandrosterone sulfate
androsta-1,4-diene-3,17-dione
Coral host
Cl
Metabolites
Endozoic.
Sample
27°C
32°C
34°C
AL
L
Endozoicomonas


a
Cholesterol incubation
E. acroporae AT381
E. acroporae Acr14
E. montiporae
26°C
34°C
Progesterone; C00410
Cholest–4–en–3–one; C00599
3–oxo–cholest–4–en–26–oate; C20839
Dehydroepiandrosterone (DHEA); C01227
5–Androstene–3,17–dione; C20252
5alpha–Androstane–3,17–dione; C00674
Androstenedione; C00280
Testosterone; C00144
Androsta–1,4–diene–3,17–dione; C20144
2–Hydroxy–cis–hex–2,4–dienoate; C11354
Hydroxyisovalerate Propionate (HIP); C20199
Control
+Cholesterol
Row-centered relative abundance


b
Testosterone incubation
E. acroporae AT381
E. acroporae Acr14
E. montiporae
26°C
34°C
Progesterone; C00410
Cholest–4–en–3–one; C00599
oxo–cholest–4–en–26–oate; C20839
Dehydroepiandrosterone (DHEA); C01227
5–Androstene–3,17–dione; C20252
5alpha–Androstane–3,17–dione; C00674
Androstenedione; C00280
Testosterone; C00144
Androsta–1,4–diene–3,17–dione; C20144
Hydroxy–cis–hex–2,4–dienoate; C11354
Hydroxyisovalerate Propionate (HIP); C20199
Control
+Testosterone
Row-centered relative abundance


c
Steroid hormone biosynthesis
Steroid degradation
Dehydroepiandrosterone
Cholesterol
5-Androstene-3,17-dione
Cholest-4-en-3-one
Pregnenolone
3-Oxo-4-cholesten-26-oate
Progesterone
5alpha-Androstane-3,17-dione
Androstenedione
Testosterone
Androsta-1,4-diene-3,17-dione
Acetyl-coA
TCA cycle
HIP
2-Hydroxy-cis-hex 2,4-dienoate
Pyruvate
Glycolysis
Chol.+E. acroporae AT381+E. montiporae
Chol.+E. acroporae Acr14
Ts.+E. acroporae AT381
Ts.+E. acroporae Acr14
Ts.+E. montiporae


d
Progesterone (m/z 315.232)
Intensity [counts] (10^6)
m/z
Testosterone (m/z 298.216)