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Metabolomic profiles of stony coral species from the Dry Tortugas National Park display inter- and intraspecies variation

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1 **Metabolomic profiles of stony coral species from the Dry Tortugas National Park**
2 **display inter- and intraspecies variation**

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16 **Running title:** Intra- and interspecies variation in coral metabolomes
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23 **Key Words:** Comparative metabolomics, Scleractinia, stony coral tissue loss disease,
24 Symbiodiniaceae, tocopherol quinones, acylcarnitines
25

Abstract:

Coral reefs are experiencing unprecedented loss in coral cover due to increased incidence of disease and bleaching events. Thus, understanding mechanisms of disease susceptibility and resilience, which vary by species, is important. In this regard, untargeted metabolomics serves as an important hypothesis-building tool enabling delineation of molecular factors underlying disease susceptibility or resilience. In this study, we characterize metabolomes of four species of visually healthy stony corals, including *Meandrina meandrites*, *Orbicella faveolata*, *Colpophyllia natans*, and *Montastraea cavernosa*, collected at least a year before stony coral tissue loss disease reached the Dry Tortugas, Florida and demonstrate that both symbiont and host-derived biochemical pathways vary by species. Metabolomes of *Meandrina meandrites* displayed minimal intraspecies variability and highest biological activity against coral pathogens when compared to other species in this study. Application of advanced metabolite annotation methods enabled delineation of several pathways underlying interspecies variability. Specifically, endosymbiont-derived vitamin E family compounds, betaine lipids, and host-derived acylcarnitines were among the top predictors of interspecies variability. Since several metabolite features that contributed to inter- and intraspecies variation are synthesized by the endosymbiotic Symbiodiniaceae, which could be a major source of these compounds in corals, our data will guide further investigations into these Symbiodiniaceae-derived pathways.

Importance.

Previous research profiling gene expression, proteins, and metabolites produced during thermal stress has reported the importance of endosymbiont-derived pathways in coral bleaching resistance. However, our understanding of interspecies variation in these pathways among healthy corals and their role in diseases is limited. We surveyed the metabolomes of four species of healthy corals with differing susceptibilities to the devastating stony coral tissue loss disease and applied advanced annotation approaches in untargeted metabolomics to determine the interspecies variation in host and endosymbiont-derived pathways. Using this approach, we propose the survey of immune markers such as vitamin E family compounds, acylcarnitines, and other metabolites to infer their role in resilience to coral diseases. As time-resolved multi-omics datasets are generated for disease-impacted corals, our approach and findings will be valuable in providing insight into the mechanisms of disease resistance.

57 **Introduction:**

58 Coral reefs are an essential component of marine ecosystems, providing habitats for nearly
59 a quarter of ocean life(1, 2), prevent shoreline erosion, and contribute to local economies(3, 4)
60 and cultural practices(5). Climate change and increases of anthropogenic stressors have resulted
61 in an unprecedented increase in incidence of coral bleaching and exposure to diseases(6-9).
62 Diseases may result in coral colony mortality and can drive local extinction of corals(10). One of
63 the biggest threats to Caribbean reefs is the continued spread of stony coral tissue loss disease
64 (SCTLD). This virulent disease spread rapidly across Florida's Coral Reef over the course of
65 several years, affecting ~22 scleractinian coral species since its first observation in 2014(11), and
66 has since spread throughout the Caribbean(12). Disease susceptibility, resilience, and lethality
67 vary significantly among affected species(11, 13-16), but underlying mechanisms behind
68 resilience remain unknown.

69 The Scleractinian (stony coral) coral holobiont is diverse, consisting of the coral animal
70 (host), the endosymbiotic dinoflagellate algae, archaea, bacteria, viruses, and fungi(17-20). Many
71 coral species are sessile organisms that rely heavily on symbiosis with photosynthetic microalgae
72 (Symbiodiniaceae) for their daily energy requirement and through associations with microbial
73 communities for nutrition and defense(17, 21-25). Within the holobiont, multiple interactions occur
74 among all members. The coral animal provides a physical habitat for the endosymbiont and a
75 diversity of microorganisms within the holobiont. Nutrient exchange between the coral host and
76 Symbiodiniaceae include the exchange of phosphorus and nitrogen (from the coral animal),
77 oxygen, and carbon (from the Symbiodiniaceae, generated through photosynthesis). Studies
78 have shown that Symbiodiniaceae can modulate intra- and interspecies susceptibility to
79 bleaching(26, 27) and disease(28). Metabolite exchange between endosymbiotic zooxanthellae
80 and bacteria influence the fitness of the endosymbiont(29). Bacteria selected by the coral
81 holobiont fulfill several key roles in holobiont metabolite cycling and pathogen defense(30).
82 *Endozoicomonas*, for example, are proposed to provide vitamins to both endosymbiotic
83 dinoflagellates and the coral host(24). The bacterial communities are uniquely structured between
84 the coral skeleton, tissue, and mucus(25). While the microbial community composition within the
85 skeleton and tissue are associated with the coral host species, environmental factors may have
86 a greater impact on the community structure within coral mucus(25). Beneficial bacteria
87 (symbionts) also produce natural products that can provide competitive advantages to the coral
88 holobiont and aid in responding to pathogens(31). Studies that profiled heat-sensitive and heat-
89 tolerant corals have established that symbiosis as well as heterotrophy play a key role in
90 determining which species thrive in the face of increasing ocean temperatures and that

91 endosymbiont identity is an important factor in survival(32-37). Although the cause of SCTLD
92 remains unknown, dysbiosis in the coral holobiont occurs with breakdown in the host-
93 endosymbiont relationship in the gastrodermis resulting in necrosis and opportunistic infections
94 by bacterial pathogens(38). There is also evidence of in situ symbiophagy (symbiont degradation)
95 by the coral host in SCTLD-exposed corals(39, 40).

96 'Omics techniques such as transcriptomics, proteomics, and metabolomics hold promise
97 for delivering insights into biochemical pathways that may drive differences in disease
98 response(41). Pairing multiple 'omics techniques enabled key insights into how *Endozoicomonas*
99 can provide key immune response related metabolites (such as vitamins) to the coral host and
100 symbiotic zooxanthellae within the coral holobiont(24). Additionally, by comparing transcriptomes
101 of stony corals of species *Acropora hyacinthus* either resilient or sensitive to bleaching stress,
102 expression of genes important for survival even under non-stress conditions in a phenomenon
103 termed frontloading was observed(42). The differential expression of orthologs related to vesicular
104 trafficking and signal transduction were positively correlated to species-specific susceptibility to
105 SCTLD(39).With advancements in data annotation strategies, untargeted metabolomics is also
106 being increasingly employed to generate, refine, and validate hypotheses to untangle interactions
107 between different members of the holobiont(43, 44). Metabolomics has been largely applied to
108 profile different genotypes of corals(45), locations(46, 47), delineate biochemical pathways
109 important in heat tolerance (48-50), and the effect of environmental factors such as use of
110 sunscreen(51). We compare the findings of these studies to the observations in this work
111 throughout our manuscript.

112 We hypothesized that metabolomes of coral species with different reported SCTLD
113 susceptibility would vary in their metabolomes, and such variations could guide future work aimed
114 at understanding of the pathways implicated in disease resilience. The sampling site in this study,
115 Dry Tortugas, Florida, was being monitored regularly in anticipation of SCTLD beginning in
116 September 2020, and the disease was first observed on May 29, 2021. Thus, the species in this
117 study were not affected by SCTLD at the time of sampling (January 2020) but are representative
118 of Florida species that are known to be susceptible to SCTLD. There are only a few investigations
119 that compare metabolic or lipid profiles of field collected corals that are SCTLD susceptible(41).
120 With the unabated spread of SCTLD along the Florida reef, opportunities to profile inter and
121 intraspecies variation in metabolomes ahead of disease and post disease was envisioned to
122 generate testable hypotheses to delineate biochemical pathways underlying disease
123 susceptibility. To test our hypothesis and compare metabolomes of coral species with different

124 SCTLD susceptibilities, we collected healthy coral fragments of four coral species, *Orbicella*
125 *faveolata*, *Montastraea cavernosa*, *Meandrina meandrites*, and *Colpophyllia natans*, ahead of the
126 SCTLD front in the Dry Tortugas. *M. meandrites* and *C. natans* are highly susceptible to SCTLD,
127 while *O. faveolata* and *M. cavernosa* are defined as moderately susceptible (13, 15, 16). SCTLD
128 susceptibility is defined by the length of time between the disease's arrival to a reef and
129 observation of lesions on a particular species, rates of lesion progression, and prevalence among
130 species (15, 16). *M. meandrites* was one of the first reported species affected in the Dry
131 Tortugas(52), while *C. natans* recruits spawned from parents in the Dry Tortugas showed *ex situ*
132 lesion progression rates of 24.9-31.1%/day, which is in range for highly SCTLD susceptible corals
133 (53). The Dry Tortugas is a unique habitat for corals along the Florida coral reef system where
134 species such as *Acropora palmata*, *Siderastrea siderea,* and *Porites astreoides* have exhibited
135 faster growth rates and enhanced reproduction relative to conspecifics in the Florida Keys (54).
136 This may be attributed to oceanographic conditions that drive periodic upwelling, which is
137 favorable for heterotrophy, and cooler temperatures and greater distance from urbanization and
138 sources of pollution(54). Exogenous untargeted metabolome profiles of Dry Tortugas' seawater
139 samples were previously reported to be distinct from the profiles of seven other zones within
140 Florida's Coral Reef prior to SCTLD arriving at the Dry Tortugas(46). Thus, the visually healthy
141 corals in this study from the Dry Tortugas provided the unique opportunity to examine and
142 compare the metabolomes of several coral species growing under optimal growth conditions in
143 Florida(54-56) before this region was affected by SCTLD.
144 In this work, we apply an untargeted high-performance liquid chromatography-mass
145 spectrometry (LC-MS)- based approach to profile the metabolomes of a small sample set of four
146 coral species (*M. meandrites*, *C. natans*, *O. faveolata*, *M. cavernosa*) utilizing recently developed
147 advanced compound annotation methods to identify metabolites underlying the interspecies
148 differences observed. While metabolomics analysis has been performed on Caribbean
149 Scleractinian corals,(43, 45, 46, 57-61) our understanding of differences between the
150 metabolomes of visually healthy Caribbean stony coral species is limited. Thus, we seek to
151 address this gap in knowledge by describing chemical classes that are variably detected between
152 four visually healthy coral species from the Dry Tortugas National Park sampled in January 2020
153 (52). In this study, we identify an endosymbiont-derived vitamin E pathway and a host-derived
154 acylcarnitine pathway that were significantly variable among species. We describe additional
155 chemical diversity by partitioning the crude extract of whole coral. Lastly, we report differences in
156 the bioactivity of partitioned extracts of whole corals against bacterial pathogens.
157

158 **Results and Discussion:**

159

160 ***Inter- and Intraspecies Variation in Metabolome Profiles**:*

161 Metabolome extracts from four stony coral species (*Orbicella faveolata*, *Monstastraea*
162 *cavernosa*, *Meandrina meandrites*, and *Colpophyllia natans*) were subjected to LC-MS analysis
163 (Figure 1, Table S1). The resulting data was analyzed using a variety of data visualization and
164 metabolite annotation tools (Figure 1C). Unsupervised principal component analysis (PCA)
165 revealed that metabolome profiles of *M. meandrites* had the lowest intraspecies variation
166 compared to the other coral species (Figure 2A). The largest interspecific separation captured
167 on the first principal component (PC) was observed between *M. meandrites* and *M. cavernosa*.
168 Four PCs captured interspecies variation between *M. meandrites* and the other species, and
169 intraspecies variation for *M. cavernosa* (Figure S1A-E). The largest intraspecies distribution on
170 PC1 was observed for *C. natans*, followed by *O. faveolata* and *M. cavernosa*. While the
171 metabolomes of *M. cavernosa* fragments were spread across PC2, tighter clustering was
172 observed for the other species along this component. PCs 3 and 4 captured metabolome variation
173 between individual extracts, revealing that additional factors beyond species are captured within
174 the metadata analysis (discussed further below) (Figure S1A-E). We calculated alpha and beta
175 diversity for each coral (using Shannon entropy and Brays-Curtis similarity metrics, respectively,
176 Figure S2) to quantify metabolome similarity. A principal coordinates analysis on the Bray-Cutis
177 similarity matrix constructed on the metabolome data revealed tighter clustering for *M.*
178 *meandrites,* while the other species were spread along the first principal coordinate (Figure S2A).
179 The within species beta diversity was significantly larger than beta diversity between species
180 (Figure S2B, Mann Whitney U Test, p=0.00082), further supporting that the metabolome analysis
181 captures variation driven by factors beyond coral species. There was no significant difference
182 between the alpha diversity for each species found using a Kruskal Wallis Test (Figure S2C, p=
183 0.119).

184 Using permutational multivariate analysis of variance (PERMANOVA) (62), we found
185 metabolome variation differed significantly across coral species ($Pseudo\text{-}F_{3,13}$ = 2.192, p=0.007),
186 with no significant effect of site (as a random effect, p=0.670). We queried whether SCTLD
187 susceptibility categorization (OFAV, MCAV = moderate; CNAT, MMEA = high) affected
188 metabolome variation. We could not examine interactive effects due to lack of Susceptibility x
189 Species replication. We performed PERMANOVA with susceptibility as a single fixed factor and
190 found that metabolome variation was significantly different between the two groups (*Pseudo-F*=
191 3.191, p=0.001). Additionally, a model with susceptibility as a fixed factor and species as a

192 random effect found that Species(Susceptibility) was slightly significantly different (*Pseudo-$F_{1,2}$* = 1.715, p=0.021). Therefore, both coral species and SCTLD susceptibility affect metabolome variation, but it is unclear how the two interact in affecting metabolome differences. We cannot fully disentangle the effects of species and susceptibility with the current sampling regime in this study, therefore such efforts are an important avenue for future inquiry. Sampling species with moderate resilience (i.e. some individuals are susceptible, others never develop lesions) where both affected and unaffected colonies were sampled would be a potential way to disentangle this effect. A non-metric multi-dimensional scaling (nMDS) plot by species was constructed to visualize metabolome variation using a method appropriate for smaller sample sizes. The nMDS plot divided the samples into four distinct clusters. Consistent with the PCA, all *M. meandrites* samples clustered together in one group, and a larger spread was observed for other species with metabolomes of *M. cavernosa* displaying the largest intraspecies variation (Figure S3). These observations aligned with previous findings where apparently healthy *M. cavernosa* from a SCTLD endemic site in Broward County showed similar intraspecies variation(43). The proximity between *M. cavernosa* colonies on the reef in this previous study explained the variation in some instances but did not completely explain the metabolome variation observed for *M. cavernosa* in Broward County(43). The beta diversity analysis conducted in this study revealed three pairs of corals from the site B or D as having the greatest Bray-Curtis similarity score to each other. These include OFAV2B/CNAT12B, MCAV6B/OFAV12B, and MMEA2D/MMEA24D. Thus, we see further evidence of reef site driving metabolome similarity in some instances, although for the MMEA pair we cannot disentangle the effect of site and species on the metabolome similarity.

213 An unsupervised hierarchical clustering analysis (HCA) revealed *M. meandrites* was the only species that clustered within a single clade (Figure 2B, purple branches), further indicating the relatively low intraspecies variation. The HCA also reveals that the corals within the aforementioned pairs identified through the Bray-Curtis similarity analysis have the greatest metabolome similarity to each other (Figure 2B). There was significant variation in metabolomic variation (multivariate dispersion) among the coral species ($F_{1,3}$=7.944, p=0.045). Overall, MCAV was the most variable (average distance to group centroid = 33.3) with the variation being significantly higher than the variation observed for MMEA (average distance = 19.2, p = 0.036). OFAV was the second most variable (average distance = 29.3) and the variation was significantly higher than the variation of MMEA (p=0.030). The relative metabolomic variation did not differ between pairwise comparisons performed for other coral species. This phenomenon has been observed in deep sea corals where interspecies differences rather than site dictated clustering of metabolite profiles(63). However, intraspecies differences may be attributable partially to site, as

226 Haydon and colleagues found metabolite differences in *Pocillopora acuta* based on reef site, even
227 after acclimation of the corals in aquaria as is the case in this study(48). Thus, when comparing
228 multiple species of corals from different reef sites, replication of species collected from each site
229 should be conducted where possible. The intraspecies metabolomic variation observed in this
230 study may further be partially explained by different genotypes(45), and both intra- and
231 interspecies variation may be influenced by the endosymbiotic profile, microbial community,
232 bleaching history, and stimuli/stressors unique to the sampling site(21, 22, 64, 65). Acquiring data
233 on seawater (exometabolomics), cataloguing abiotic factors at reef sites, and profiling the
234 endosymbiont and microbial community will aid in disentangling what additional factors drive
235 metabolome variation. In a metabolomics study of cultured *Symbiodinium* species, Klueter *et al*
236 noted that metabolite profiles varied by species and the degree of metabolome variation was not
237 ubiquitous across species given the different classification error rates of each symbiont
238 species(66). The distinct metabolome profiles of *M. meandrites* compared to the other corals
239 species in this study could be influenced by the symbiont types of the coral species. The *M.*
240 *meandrites* sampled for this study may host symbionts with highly similar metabolomes or
241 interaction networks with the associated microbiome, while the other coral species may host
242 symbionts and/or microbiome with more diverse metabolomes. Incorporating microbiome
243 analysis and symbiont typing into metabolome studies would be beneficial towards delineating
244 how the degree of observed metabolome variation may correspond to the symbiont and the
245 microbiome species present. Another factor that may contribute to the intraspecies metabolome
246 variation captured in this study is cryptic lineages observed within the studied species(67). Cryptic
247 coral species lineages may share phenotypic traits but have distinct underlying genomic
248 differences(67). *M. cavernosa*(68, 69) and *O. faveolata*(70, 71) are reported to have cryptic
249 lineages. The genomic differences between the coral hosts imply a strong possibility for
250 metabolite differences (since the metabolome reflects the functional biochemical state of the
251 system, in this case the coral holobiont). Proven association with different symbiont genera
252 (reported for *O. faveolata*(70)) and potential association with different microbial assemblages (yet
253 to be studied for the species in this report) among cryptic lineages could further diverge
254 metabolome profiles, as all the members of the coral holobiont influence and contribute to the
255 metabolome. Such phenomena(67) may well explain the metabolome variation of *M. cavernosa*
256 and *O. faveolata* in this study, and although cryptic lineages have not yet been identified for
257 *Colpophyllia* (67) the observed metabolome variation in *C. natans* may be partially explained by
258 this as well.

259 The UpSet Plot generated to show the distribution of metabolite features revealed the
260 greatest number of unique features were detected in *M. meandrites* extracts, followed by *M.*
261 *cavernosa*, *C. natans*, and *O. faveolata* (Figure 2C). Since the statistical analyses revealed a
262 distinct metabolome profile for *M. meandrites* and a greater intraspecies metabolite variation
263 captured for the other coral species, unique features present in *M. meandrites* and features that
264 were variably detected among *M. meandrites* and other coral species were prioritized for
265 annotation and are described below.

266 ***Metabolite Features Driving Variation***

267 **Vitamin E family compounds as potential biomarkers of stressor susceptibility:**

268 A metabolite feature *m/z*_RT (*m/z*: mass to charge, RT: retention time in min)
269 449.398_21.3 min, uniquely detected in *M. meandrites* extracts, was proposed as α-
270 tocopherolhydroquinone by the in silico annotation tool MolDiscovery. SIRIUS with CSI:FingerID
271 also proposed the annotation for this feature as α-tocopherolhydroquinone. We searched for α-
272 tocopherolhydroquinone spectra in the literature and used MS^2 spectral matching with a published
273 spectrum(72) of silicated α-tocopherolhydroquinone to further support the annotation (Figure
274 S4A). This feature clustered in Feature Based Molecular Networking (FBMN) analysis with
275 another feature at 447.383_21.3 min representing one unsaturation from α-
276 tocopherolhydroquinone ($\Delta m/z$= 2.015). We annotated this feature as α-tocopherolquinone, the
277 oxidation product of α-tocopherolhydroquinone(73, 74) and confirmed this annotation with an
278 analytical standard of α-tocopherolquinone (Figure S4B-C). To identify additional metabolites we
279 applied unsupervised substructure discovery using MS2LDA(75) (Figure 3A, Table S2).

280 It is interesting to note that α-tocopherolquinone, an oxidation product of α-tocopherol(76,
281 77), and α-tocopherolhydroquinone were exclusively detected in highly SCTLD susceptible *M.*
282 *meandrites* and *C. natans*, but not in *O. faveolata* and *M. cavernosa* with moderate SCTLD
283 susceptibility(15, 16). These features were also not detected in the extracts of cultured
284 Symbiodiniaceae included in this study (Figure 3B). The *Durusdinium*-associated mangrove coral
285 *Pocillopora acuta* was previously reported by Haydon and colleagues to accumulate α-tocopherol
286 in summer(48). This observation was suggested as a possible mechanism of 'frontloading'
287 associated with the resilience of *Durusdinium*-associated corals (48). Tocopherols are the most
288 prominent antioxidants that counteract lipid peroxidation. Using transcriptomics, the gene for
289 arachidonate 5-lipoxygenase (ALOX5), was found to be significantly differentially expressed with
290 its highest expression in the most susceptible corals, including *C natans*(39). ALOX5 is a key
291 enzyme in mediating lipid peroxidation(78) which can lead to cell death such as apoptosis,
292 ferroptosis, and pyroptosis(78). Thus, we searched the literature to identify studies that might link

293 α-tocopherol(hydro)quinones with lipid peroxidation and cell death. While linking specific
294 metabolites with processes is outside the scope of this study, we can speculate on possible
295 functions of metabolites and determine future avenues of inquiry based on literature precedence.
296 Indeed, a recent report updated the mechanism-of-action for iron-dependent anti-apoptotic
297 activity (ferroptosis) of α-tocopherol(79). Tocopherol was suggested to be the pro-vitamin E form,
298 while the (hydro)quinone forms produced from oxidation of α-tocopherol were shown to be the
299 activated forms responsible for prevention of cell death(79). Thus, it is possible that our detection
300 of the α-tocopherol(hydro)quinones indicates the coral cells are frontloading the activated form of
301 vitamin E, which is counteracting lipid peroxidation resulting in detection of α-
302 tocopherol(hydro)quinones.

303 When corals were previously challenged with bacterial pathogen-associated
304 lipopolysaccharides, susceptible corals demonstrated a transcriptome response related to
305 apoptosis, while resistant corals transcribed genes related to autophagy, a more modulated
306 response to stressors(80). The damage threshold hypothesis proposes that coral disease
307 susceptibility is inversely related to the upper limit of damage a coral can withstand before harmful
308 effects are observed(81). Corals with a low damage threshold (high susceptibility) may be unable
309 to modulate immune responses; either mounting too high of a response, leading to auto-immune
310 challenges, or too low of a response before cellular death is imminent. Thus, the varied detection
311 of tocopherol(hydro)quinones in this study should be further investigated to determine if these
312 metabolites serve as a biomarker of corals particularly susceptible to disease, represent stressor
313 history, and if they vary temporally with disease progression.

314 *M. meandrites* and *O. faveolata* had the highest relative abundance of α-tocomonoenol
315 (Figure 3A). α-tocomonoenol was previously detected at higher abundance in apparently healthy
316 *M. cavernosa* compared with diseased corals(43). The analogue α-tocotrienol (*m/z* 424.333) with
317 three degrees of unsaturation, was exclusively detected in SCTLD-affected *M. cavernosa*, while
318 other unsaturated analogs were likewise detected at higher abundance in the diseased corals(43).
319 In this study, where we have analyzed healthy corals ahead of the SCTLD front, α-tocotrienol was
320 not detected. Based on these results, we hypothesize that tocotrienols may serve as biomarkers
321 for coral disease, wherein accumulation coincides with disease progression. A time course study
322 that tracks how tocotrienol analogues and tocopherolquinones accumulate in response to disease
323 exposure is required to validate this hypothesis. Recent work highlighting differential detection of
324 tocopherol upon heat stress among resilient and susceptible species(48, 82) and our work
325 reporting detection of different tocopherol analogues among healthy and SCTLD-affected coral
326 colonies suggest that this endosymbiont pathway likely plays an important role in coral health and

327 resilience(48, 83); warranting studies that monitor tocopherol-related metabolite production over
328 time after disease exposure.

329 **Acylcarnitine profiles differentiate *Meandrina meandrites*:**

330 Feature 476.373_14.4 min was proposed by SIRIUS with CSI:FingerID as
331 docosatetraenoyl carnitine (C22:4) (Figure 4A, B). To confirm this annotation prediction and to
332 determine if other acylcarnitines were present in our data, the output of the MS2LDA analysis was
333 consulted. This feature shares MS2LDA substructure motif 185 with feature 276.180_2.8 min,
334 which was annotated as hydroxyhexanoyl carnitine (C6:0-OH) based on the MS^2 fragment peak
335 at *m/z* 217.107 associated with the neutral loss of trimethylamine ($\Delta m/z$= 59.07, Figure 4C, S4A).
336 A variety of acylcarnitines were further annotated with the aid of the GNPS spectral library,
337 MassQL, substructure motif 185, and SIRIUS with CSI:FingerID (Figure 4, S4B-O, Table S2).

338 Acylcarnitines are typically host-derived metabolites, and these metabolites were not
339 detected in the cultured Symbiodiniaceae extracts in this study. Acylcarnitines have been
340 detected at higher abundances in the daytime exometabolomes of *Porites* and *Pocillopora*
341 compared to algae (turfing microalgae, macroalgae, and crustose coralline algae), where they are
342 hypothesized to play a role in nitrogen and phosphorous cycling(84). Acylcarnitines play an
343 integral role in metabolism of fatty acids in mitochondria(85) and maintenance of available pools
344 of free coenzyme A(86). In the diatom *Phaeodactylum tricornutum*, propanoyl-carnitine and
345 butanoyl-carnitine accumulate under nitrogen-starvation(87). Accumulation of acylcarnitine
346 concentrations have been linked with cell toxicity(88), mitochondrial dysfunction(89-91), and
347 dysfunction in cellular bioenergetics in humans(88). Acylcarnitines have been found to be
348 upregulated in corals upon exposure to octocrylene, an ingredient used in sunscreens(51), which
349 is the only study reporting conditional dysregulation of acylcarnitine levels in corals found in our
350 literature search. In this study, the interspecies variation of all features annotated as acylcarnitines
351 were analyzed with a Kruskal Wallis test with Dunn's post-test (adjusted p<0.05). The
352 acylcarnitines with fatty acyl tails with C13-C20 are classified as long chain, and tails >C21 as
353 very long chain(92). Features that were differentially detected showed two interspecies patterns
354 based on the acyl chain length (Figure 4B, C, F-J and S4D-O). The hydroxyhexanoyl acylcarnitine
355 and very long chain acylcarnitines were detected at higher intensity in *M. meandrites* (Figure 4 B,
356 C, F-I). The accumulation of long chain acylcarnitines is associated with several metabolic
357 diseases in humans(92). Differences in acylcarnitine profiles have also been reported as
358 indicators of frailty in humans(93). Given that certain acylcarnitine analogues are detected at
359 higher intensity in *M. meandrites* (Figure 4 and S4), a highly SCTLD-susceptible species, it is
360 possible that acylcarnitine profiling could represent disease history and/or higher susceptibility to

361 disease. Interestingly, when an HCA was performed on only the annotated acylcarnitine features,
362 a clear separation of *M. meandrites* from other coral species was observed (Figure 4K). Thus,
363 host-derived acylcarnitines display a species-specific profile. Since several acylcarnitines were
364 variably detected in these apparently healthy corals, the understudied role of carnitines in disease
365 resilience and susceptibility in corals should be further investigated.

366 Several unknown acylcarnitines were distributed differentially across the coral species
367 (Figure 4J and S4G-O). Upon manual inspection of fragmentation spectra, we propose the
368 annotation of these features as acylcarnitines containing fatty acid esters of hydroxy fatty acids
369 (known as FAHFAs) (Figure S5A, Table S2). The fragments at *m/z* 85.028 and 144.102, presence
370 of a fragment corresponding to hydroxylated fatty acid of acylcarnitine (CAR 14:1-OH), and the
371 presence of an additional fatty acid tail fragment (C18:0) supported the annotation of an FAHFA-
372 containing acylcarnitines (Figure S5A, bottom spectrum; CAR 14:1-(O-18:0)). FAHFAs are a
373 conserved class of lipids that are widely reported, including in dietary plants(94, 95), as defense
374 molecules in caterpillars named as mayolenes (96, 97), as anti-inflammatory metabolites in
375 humans(98), and in the corallivore Crown-of-Thorns Starfish(99). Oxidative and environmental
376 stress increase the synthesis of FAHFAs and ornithine-conjugated FAHFAs(100, 101).
377 Acylcarnitines containing FAHFAs have not been previously reported and warrant further
378 investigation for structural characterization and their role in coral biology. We searched for these
379 acylcarnitines features in the publicly available datasets on the MassIVE server using
380 MASST(102). These features were found in several marine organism-derived datasets including
381 datasets from several coral species (Table S3) but were not observed in human-derived datasets.
382 These observations further strengthen the role and application of modern methods in data
383 analysis in untargeted metabolomics to discover biologically relevant metabolic pathways and
384 generate testable hypotheses. Here, access to public datasets on these pristine endangered coral
385 species is advantageous.

386 **DGCC betaine lipids with 16:0 fatty acyl tails are differentially detected between species:**

387 Several differentiating features were identified as diacylglyceryl-
388 carboxyhydroxymethylcholine (DGCC) betaine lipids. Feature 774.584_19.6 min was a GNPS
389 library match to DGCC(36:5) (Figure 5A, Figure S6A). The fragment peaks at *m/z* 490.373 and
390 472.363 in the MS^2 spectra, which are characteristic of the chemical substructure containing a
391 16:0 fatty acyl tail, enable further annotation of this feature as DGCC(16:0_20:5) (Figure S7). This
392 feature was variably detected among coral species, present at highest abundance in *M.*
393 *cavernosa*. As expected, the feature 490.373_13.1 min, annotated as lyso-DGCC(16:0) known to
394 be a constituent of healthy corals(43, 58, 103), was detected in all species (Figure 5B, Figure

395 S6B). We used MassQL to search for additional DGCC analogues containing a 16:0 fatty acyl tail
396 (Figure S6C). This approach permitted the annotation of additional metabolite features, detected
397 at highest abundances in *M. meandrites*, as lyso-DGCC(16:0) analogues (Figure 5 and S7, Table
398 S2). Diacylated and unsaturated DGCC betaine lipids have been previously proposed as
399 biomarkers of coral bleaching(58, 103). The increase in lipid unsaturation is suggestive of
400 increased cell death when the antioxidative capacity of cells is lowered(104). DGCC betaine lipids
401 are biosynthesized by Symbiodiniaceae(50). We searched the metabolite data acquired on
402 cultured Symbiodiniaceae for the presence of the annotated DGCC analogues. While
403 monoacylated lyso-DGCC(16:0) was detected in all cultured Symbiodiniaceae genera, the
404 diacylated analogues were notably absent in *Durusdinium* extracts, the genera known to be most
405 thermotolerant(105-108) (Figure 5H). Roach *et al*. noted a higher abundance of lyso-DGCCs in
406 historically non-bleached corals, while unsaturated and DGCCs were abundant in historically
407 bleached corals(58). Rosset *et al*. observed significantly higher abundance of lyso-DGCC and
408 unsaturated DGCCs in thermotolerant *D. trenchii* as compared to *Cladocopium* C3 in both control
409 and heat stressed conditions(49, 50). Symbiodiniaceae genera show differential responses to
410 thermal and irradiance stress, which affects the entire holobiont response to stressors(64, 109-
411 111). The variable detection of the DGCC(16:0) analogues in the coral extracts may indicate
412 variable bleaching history or the presence or absence of certain Symbiodiniaceae species in the
413 coral colonies sampled. Previous reports suggest that the DGCC lipid profile is influenced by the
414 host(112). Given that algae transform their membranes in response to a variety of stimuli and
415 stressors(113-115), it is also possible that the variable detection of the diacylated DGCC(16:0)
416 analogues is reflective of host-dependent shifts in betaine lipid profiles of Symbiodiniaceae.

417 **Carotenoid pigments do not show coral species-specific patterns:**

418 Carotenoids are important antioxidants in photosynthetic organisms. Symbiodiniaceae
419 produce several carotenoids such as peridinin, fucoxanthin, astaxanthin, diatoxanthin,
420 diadinoxanthin, and neoxanthin, with peridinin being the most prevalent and abundant(116, 117).
421 We examined whether endosymbiont-derived pigment profiles contributed to variation among the
422 coral colonies analyzed in this study. Several pigments were annotated using mass spectral
423 search and literature search (Figure 6 and S8A-F, Table S2). The features annotated as pigments
424 were also analyzed by HCA (Figure 6B). The pigment profile did not display clear interspecies
425 variation but did display intraspecies variation. Peridinin was detected in all coral extracts, while
426 fucoxanthin was detected in only a few coral extracts (Figure 6A). Among cultured
427 Symbiodiniaceae in this study, peridinin was detected in all genera whereas fucoxanthin was only
428 detected in thermotolerant *Durusdinium* cultures (Figure S8G). Since Wakahama *et al*. reported

429 a negative correlation between presence of fucoxanthin and peridinin in a variety of symbiotic and
430 free living *Symbiodinium* strains(118), we confirmed detection of fucoxanthin in peridinin-
431 containing coral extracts using an analytical standard of fucoxanthin (Figure S8F). Detection of
432 both pigments may represent presence of multiple strains of Symbiodiniaceae within the cultures.

433 **Butanol partitions of whole coral extracts enable additional metabolite annotations**

434 The crude extracts from the whole coral samples were further partitioned into ethyl acetate
435 (EtOAc) and butanol (BuOH) solvents and the bioactivity of these fractions was tested against the
436 potential SCTLD-associated pathogens *Vibrio coralliilyticus* OfT6-21 and OfT7-21, *Leisingera* sp.
437 McT4−56, and *Alteromonas* sp. McT4-15(119, 120) using an agar disk-diffusion assay (Figure
438 7A, Figure S9A). Partitions only exhibited activity against *V. coralliilyticus* strains. The largest
439 zones of inhibition were observed for BuOH partitions of *M. meandrites* against both pathogens
440 (Figure 7A and S9A). Thus, untargeted metabolomics data were acquired on BuOH partitions of
441 all species. The metabolite data was analyzed following the scheme outlined in Figure 1. Within
442 the BuOH partitions, an additional 560 metabolite features were detected (Figure 7B). The UpSet
443 Plot analysis showed the greatest number of unique features was detected in *C. natans* extracts,
444 followed by *M. cavernosa*, *M. meandrites*, and *O. faveolata* (Figure 7B). We used CANOPUS to
445 predict the chemical classes of these features (Table S4). For the features uniquely detected in
446 the BuOH partitions, none of the metabolites in the CANOPUS-predicted natural product
447 pathways were significantly enriched in *M. meandrites* compared to the other species (Figure 7C).
448 The UpSet Plot and CANOPUS output were used to guide compound annotations (Figure 7D).

449 A feature, detected exclusively in BuOH partitions at 280.157_7.9 min was annotated as
450 Tau-C10:0 based on MS^2 spectral matching (Figure S10A). We also observed the presence of
451 the *N*-acyl taurines in several publicly available datasets acquired on diatoms, dinoflagellates,
452 and seawater by searching the MS^2 spectrum of this metabolite in MASST (Table S3). *N*-acyl
453 taurines have been implicated as important signaling molecules in several human processes
454 including postprandial glucose regulation(121), but these molecules have not been previously
455 described in corals. Thus, partitioning crude extracts into organic solvents can enable detection
456 and characterization of low-abundance metabolites, which are otherwise below the limit of
457 detection. Feature 267.960_5.3 min with an isotopic pattern of a brominated compound was
458 uniquely detected in the BuOH partitions of *O. faveolata*. This annotated as caelestine A based
459 on MS^2 spectral matching and MS^1 isotopic pattern (Figure S10B-C). CANOPUS predicted the
460 chemical class as hydropyrimidine carboxylic acids and derivatives. Caelestine A, a brominated
461 quinoline carboxylic acid, has been reported as a possible indicator of a response to heat stress
462 in the invasive bryozoan *Bugula neritina*(122). A feature at 615.346_5.9 min was proposed as a

463 tunicyclin G analogue by DEREPLICATOR, which compares experimental MS^2 spectra against
464 predicted in silico MS^2 spectra of peptides(123) and CANOPUS predicted the chemical class of
465 this feature as oligopeptides. We putatively annotated the feature 615.346_5.9 min as a
466 polypeptide with partial sequence NGAI/LA (Figure S9C). This polypeptide was detected in *M.*
467 *cavernosa* and *O. faveolata* BuOH partitions. Although we could not link the enhanced
468 antibacterial activity with specific molecules or chemical classes due to a small sample size, these
469 annotations show that partitioning of whole coral metabolomic extracts increases the breadth of
470 detected metabolites and enables annotation of low-abundance natural products. Annotation of
471 individual metabolites is still a tedious and manual task. As spectral libraries are populated by the
472 community, and in silico compound annotation methods advance, these datasets will be a
473 valuable resource to untangle mechanisms of symbiosis between members of coral holobionts.
474 **Conclusion:**
475 The visually healthy corals collected from the Dry Tortugas, a region of Florida with
476 oceanographic conditions that support coral productivity, revealed interspecies metabolome
477 differences. Tight clustering of the *M. meandrites* metabolome indicated similar metabolite
478 profiles, while higher metabolome variation was found for *C. natans*, *M. cavernosa*, and *O.*
479 *faveolata*. Metabolites driving the variation between species included tocopherol(hydro)quinones,
480 diacylated betaine lipids, and acylcarnitines. This is the first report describing differences in
481 acylcarnitine profiles between coral species and the discovery of potentially novel analogues
482 containing an additional fatty acid group. Given the specificity of these acylcarnitine-FAHFA to
483 only marine organisms based on the MASST search, the biochemical function of these molecules
484 is of particular interest. The role of acylcarnitines in cellular energetics is well established; the
485 varied detection of acylcarnitines in corals may indicate variability among species in their ability
486 to readily utilize these pathways. Future work will focus on structural description of these
487 carnitines. How the profiles of metabolites reported in this manuscript change over time should
488 also be characterized to determine their viability as biomarkers of health, disease, and lesion
489 progression. The juxtaposition of *M. meandrites* SCTLD susceptibility and the observed highest
490 bioactivity of the BuOH partitions of the extracts of this species against putative secondary SCTLD
491 pathogens generates interesting avenues for future study, including how molecular dynamics of
492 pathogen response and disease susceptibility might explain discrepancies between disease
493 susceptibilities in the field while metabolite extracts show high antibacterial activity when
494 challenged with in lab-assays to a specific pathogen. Additional studies can also incorporate
495 knowledge of environmental factors like heat stress to determine how biochemical disease
496 dynamics and susceptibility may shift in the field.

497 As SCTLD appears to affect Symbiodiniaceae and disrupt their relationship with the
498 host(13, 38, 40, 43), it is imperative to understand the differences in chemical cross-talk between
499 the corals and endosymbionts. Symbiodiniaceae dynamics within the host (e.g., relative
500 abundance, density, species) will likely have an effect on the metabolomic profiles. In this study,
501 several Symbiodiniaceae metabolites driving interspecies differences and endosymbiont-derived
502 carotenoid pigments displayed both inter- and intraspecies variation suggesting the presence of
503 different endosymbiont genotypes in these samples and/or different endosymbiont-host dynamics
504 among species. Thus, metabolomic studies on Symbiodiniaceae directly isolated from field corals
505 will enable source tracking to tease apart host-derived, diet-derived and endosymbiont-derived
506 compounds. Given that the endosymbiont fraction can be isolated from corals using mechanical
507 methods such as the air brush technique (124), we propose future studies also include
508 comprehensive metabolic profiling of these endosymbionts prior to and upon exposure to disease.
509 Such differences should be interrogated across corals with different disease susceptibility and
510 with different endosymbiont profiles. Furthermore, studies of metabolomic profiles taken at
511 discrete time-points after disease exposure will provide insights into the transitory response of
512 corals to disease stressors. With the increased application of untargeted metabolomics methods
513 to study coral physiology, availability of annotated datasets, and our ability to mine public datasets
514 using methods such as MASST, discoveries pertaining to chemical interactions between the coral
515 host, the endosymbiont, environment, and the microbiome that define health status are a real
516 possibility. By advancing our knowledge of the biochemical pathways involved in coral health and
517 susceptibility to disease, we can disentangle the sources of metabolites, and how they change
518 with time and increasing anthropogenic and climate related stressors.
519
520 **Materials and Methods:**
521 **Coral Sample Collection and Extraction Procedure.** Whole coral fragments of a maximum size
522 of 200 cm^2 were collected on SCUBA from four visually healthy Scleractinia coral species, *O.*
523 *faveolata* (n=4), *M. cavernosa* (n=4), *M. meandrites* (n=4), and *C. natans* (n=3). These were
524 collected in January 2020 from four sites outside of the Dry Tortugas National Park (Figure 1 and
525 Table S1). This collection occurred ahead of the SCTLD front, which was first observed at the Dry
526 Tortugas in May 2021(125). Collection also occurred during a time of year when temperature
527 stress and any associated paling or bleaching of the corals should not have been occurring, and
528 none was observed at time of collection. Corals were chiseled at the base until they released from
529 the substrate and then were transported back to the diving vessel in 18.9 L plastic bags filled with
530 ocean water. Collected corals larger than 25 cm^2 were cut down to this size on the diving vessel

531 using an AquaSaw (Gryphon C-40 CR). The cut portions and whole colonies were stored in a
532 1000 L covered insulated container (Bonar Plastics, PB2145) filled halfway with ocean water. Air
533 stones within the container allowed for aeration and water movement. A complete water change
534 was performed on the container four times daily. Collections were conducted over two days before
535 corals were transported the morning of the third day. The cruise was sponsored by the Florida
536 Department of Environmental Protection and sample collection was covered by permit FKNMS-
537 2019-160 to Valerie Paul. All corals collected from the field were transported to the Smithsonian
538 Marine Station in Fort Pierce, FL. For transport, individual colonies were wrapped in plastic bubble
539 wrap moistened with ocean water and then placed in a cooler.

540 Upon arrival, corals were rinsed with filtered seawater (FSW) and stored in a large indoor
541 recirculating system holding approximately 570 L of FSW at 25.5 °C ± 0.3 °C. The FSW was
542 collected from an intake pipe extending 1600 m offshore South Hutchingson Island, Port Saint
543 Lucie, FL and was filtered progressively through 20, 1.0, 0.5, and 0.35 µm pore filters. While
544 stored prior to use in the recirculating system, the FSW constantly circulated through a 20 µm
545 pore filter, a filter canister with ROX 0.8 aquarium carbon (Bulk Reef Supply), and a 36-watt Turbo-
546 twist 12x UV sterilizer (Coralife) in series. The recirculating system contained a UV sterilizer (same
547 model as described), two circulating pumps (AquaTop MaxFlow MCP-5) to create water
548 movement, and a row of 6 blue-white 30 cm^2 LED panels (HQPR) to create 150 to 250 µmol
549 photons m^{-2} sec^{-1} for the captive corals. Corals were stored in the recirculating system for 5 days
550 prior to sampling to allow them time to recover after transport, with a partial water change on the
551 fourth day. All corals were held together in a single table.

552 After the fifth day, the corals were cut into smaller segments with a rock saw, and the
553 blade was constantly sprayed down with UV/filter-sterilized seawater to cool the blade and wash
554 off any debris, thus reducing cross-contamination between corals. The coral fragments ranged
555 from 1-13 cm^2 in surface area (6.2+3.7 cm^2, mean+SD) were immediately frozen (-80 °C) and
556 lyophilized the next day. Coral fragments were lyophilized overnight and then extracted twice
557 using a 2:2:1 mixture of ethyl acetate (EtOAc), methanol (MeOH) and water (H_2O) at room
558 temperature in 20 mL scintillation vials. For the extraction, coral fragments were covered with an
559 excess of solvent mixture, sonicated for 5-10 min and left to sit for 3 h on the initial extraction and
560 overnight for the second extraction. The liquid extract was then transferred into a round-bottom
561 flask using filter paper to prevent the transfer of coral fragments. The coral extract was then dried
562 via rotary-evaporation (Buchi Rotovapor R-210) in a 35 °C water bath (Buchi Heating Bath B-491)
563 and weighed to determine the amount of crude extract. The extracts were dried *in vacuo* and 0.5

564 mg of the extract was transferred to Eppendorf tubes for metabolomics data analysis. The extracts
565 were stored at −20 °C until UPLC-MS data was acquired.

566 **Endosymbiont Metabolome Data**

567 We previously reported on metabolome profiles of Symbiodiniaceae isolates provided by
568 Mary Alice Coffroth from the University of Buffalo Undersea Reef Research (BURR) collection
569 (43). Given the challenges involved in isolating and culturing Symbiodiniaceae, we used this
570 publicly available data(43) (massIVE identifier MSV000087471) in this current study to aid in
571 determining the biosynthetic producer of detected metabolites. Briefly, the endosymbionts were
572 isolated by Mary Alice Coffroth from Orbicella faveolata corals sampled between 2002-2005 from
573 the Florida Keys. Isolate extracts were sent by Richard Karp and Andrew Baker, (University of
574 Miami). Culture conditions included incubation at 27°C in F/2 media, with 20 µE of light on a 14:10
575 diurnal cycle. Extracts of the culture were performed as previously described(43), using 2:2:1
576 EtOAc:MeOH:H_2O to extract pelletized cellular cultures. Solvents were removed and the dried
577 samples were transferred in 3:1 MeOH:H_2O into a 1.5 mL microcentrifuge tube. After
578 centrifugation, the supernatant was transferred to a microcentrifuge tube and removed via
579 SpeedVac for 3 h. The extract was frozen, lyophilized, analyzed using UPLC-MS/MS. Please see
580 Deutsch *et al* 2021 for detailed methodology(43).
581

582 **Mass Spectrometry Data Acquisition and Analysis.** The dried extracts were resuspended in
583 100% MeOH containing 1 µM sulfadimethoxine as an internal standard. The samples were
584 analyzed with an Agilent 1290 Infinity II UHPLC system (Agilent Technologies) using a Kinetex
585 1.7 µm C18 reversed phase UHPLC column (50 × 2.1 mm) for chromatographic separation,
586 coupled to an ImpactII ultrahigh resolution Qq-TOF mass spectrometer (Bruker Daltonics, GmbH,
587 Bremen, Germany) equipped with an ESI source for MS/MS analysis. MS/MS spectra were
588 acquired in positive mode as previously described(43). Metabolomics data on the cultured
589 Symbiodiniaceae from the Burr Collection was previously acquired(43). The strains utilized are
590 reported in Table S5.

591 The raw data was converted to mzXML format using vendor software. MZmine 2.53 was
592 used to extract metabolite features with steps for mass detection, chromatogram building,
593 chromatogram deconvolution, isotopic grouping, retention time alignment, duplicate removal, and
594 missing peak filling(126). This processed data was submitted to the feature-based molecular
595 networking workflow on the Global Natural Product Social Molecular Networking (GNPS)
596 platform(127). The output of MZmine includes information about LC-MS features across all

597 samples containing the *m/z* value, retention time, the area under the peak for the corresponding
598 chromatogram, and a unique identifier for each feature. The quantification table and the linked
599 MS^2 spectra were exported using the GNPS export module(126, 128) and the SIRIUS 4.0 export
600 module(126, 129). Feature Based Molecular Networking was performed using the MS^2 spectra
601 (.mgf file) and the quantification table (.csv file). The molecular network was generated as
602 previously described(43). The molecular network and the generation parameters are available
603 here. The molecular network was visualized using Cytopscape v3.7.2(130). The MS2LDA
604 analysis was performed as previously described with default parameters(131, 132). The MassQL
605 Sandbox Dashboard(133) (v 0.3) on the GNPS platform was used to construct the spectral pattern
606 queries for the MassQL search. Feature annotation was performed using SIRIUS with
607 CSI:FingerID, MolDiscovery(134), GNPS spectral library matching, MassQL, MASST, and
608 literature searches. The metabolite annotations presented herein follow the "level 2" annotation
609 standard based upon spectral similarity with public spectral libraries, spectra published in the
610 literature, and through spectral comparison with the analytical standards as proposed by the
611 Metabolomics Society Standard Initiative(135). All mzXML files included in this study can be
612 accessed publicly on the repository Mass Spectrometry Interactive Virtual Environment
613 (MassIVE) with ID MSV000089633. The commercial analytical standard for α-tocopherolquinone
614 (catalog number 35365) was purchased from Cayman Chemical Company and the commercial
615 analytical standard for fucoxanthin (catalog number 16337) was purchased from Sigma Aldrich.

616 Prior to statistical analysis, blank subtraction was performed as previously described(43)
617 to filter out features detected in the solvent and media controls. Unsupervised multivariate
618 statistical analyses including principal component analysis(136) and hierarchical clustering
619 analysis(137) were performed using MetaboAnalyst 5.0(138) and pareto scaling was employed
620 prior to the analyses. The Plotter Dashboard (v.0.5) on the GNPS platform was used to construct
621 boxplots for metabolite features of interest. A nonparametric Kruskal Wallis test with Dunn's
622 posttest was performed in R studio. The UpSet Plots were made using the Intervene app(139).

623 To test for an effect of coral species on metabolomic variation, we used a permutational
624 multivariate analysis of variance (PERMANOVA)(62). Coral species was treated as a fixed
625 effect (four levels), with site included as a random nested effect (four levels). The PERMANOVA
626 was based on a Bray-Curtis similarity matrix(140), type III (partial) sums of squares, and 999
627 random permutations of square-root transformed data (to down-weight heavily dominant
628 variables) under a reduced model. Both PERMANOVA and non-metric multidimensional
629 scaling(141) plot were constructed from a Bray-Curtis similarity matrix of square root transformed
630 data, which was performed using PRIMER v7(142). To quantify metabolomic variation within and

631 between coral species, we calculated their multivariate dispersion using the PERMDISP routine
632 (143). PERMDISP calculates the distance of each observation (in this case each coral sample)
633 to its group centroid (in this case each coral species) and then compares the average of these
634 distances among groups. It is a multivariate extension of Levene's test, with the p-values obtained
635 using permutations of the raw data. This allowed us to make inferences about the relative size of
636 the clouds in multivariate space within and between coral species. The tests were based on the
637 same transformed data and Bray-Curtis similarity matrix as our PERMANOVA tests. Shannon
638 entropy(144) was calculated for the alpha diversity metric using a Jupyter Notebook. A Bray-Curtis
639 similarity matrix of log-transformed data was constructed using Primer v7 for the beta diversity
640 metric. A principal coordinates analysis(145) was constructed on the Bray-Curtis similarity matrix.
641 Several in silico tools were used to aid in metabolite annotations. MolDiscovery compares
642 in silico generated MS^2 spectra of small molecules to user-uploaded experimental MS^2
643 spectra(134). SIRIUS computes putative chemical formulas based on user-uploaded MS^1 isotopic
644 peaks and MS^2 fragmentation patterns(129). CSI:FingerID transforms MS^2 spectra into predicted
645 structural fingerprints that enable matching to fingerprints generated from structure
646 databases(146). CANOPUS, which predicts the chemical class of metabolites by utilizing
647 CSI:FingerID's predicted structural fingerprints, proposed the chemical class of 449.398_21.3 min
648 as Vitamin E compounds(147). Unsupervised substructure discovery performed through
649 MS2LDA(148) enabled annotations of several classes. Substructure motif 108 consisted of
650 fragmentation peaks characteristic of tocopherol substructure (Figure S3D). Motif 185 containing
651 characteristic carnitine headgroup fragment peaks(91, 149) at *m/z* 85.028 and 144.102 (Figure
652 4D) aided acylcarnitine annotations. Supervised substructure discovery was performed using
653 MassQL, a MS query language platform that outputs metabolite features based on sets of user-
654 defined fragment peaks and neutral losses(133).

655 **Extract Partitioning of Crude Extracts of Coral Metabolomes.** Crude coral extracts were
656 partitioned to remove salts and separate compounds based on polarity. First, 3 mL of EtOAc was
657 added to 20 mL scintillation vials containing dry crude extracts. Vials were sonicated to resuspend
658 the extracts for 30-60 seconds. 3 mL of H_2O and another 1 mL of EtOAc was then added and the
659 vials swirled to mix. Vials were then left to separate into distinct layers. The EtOAc layer was
660 transferred via glass pipette into a clean and pre-weighed 20 mL scintillation vial. An additional 2
661 mL of EtOAC was then added to the crude mixture with water, swirled to mix and left to separate
662 again. The EtOAc layer was again transferred into the vial containing the EtOAc partition. The
663 EtOAc partitions were then dried via a SpeedVac vacuum concentrator (Thermo Scientific Savant

664 SPD121P) at 35 °C. The remaining aqueous extract was then partitioned using n-butanol (BuOH).
665 Approximately 2 mL of BuOH was added to the aqueous extract, swirled to mix and then left to
666 sit until distinct layers formed. The BuOH partition was then transferred into a clean and pre-
667 weighed 20 mL scintillation vial. Another round of BuOH partitioning was performed by adding an
668 additional 1 mL of BuOH to the aqueous extract, mixing and allowing time for a final separation.
669 The BuOH layer was transferred to the vial containing the initial BuOH partition, which was then
670 dried via rotary-evaporation and analyzed using UPLC-HRMS. The BuOH partitions were
671 resuspended and LC-MS data was acquired and analyzed following the procedure outlined in
672 "Mass Spectrometry Data Acquisition and Analysis".

673 **Disk Diffusion.** Coral extracts were tested for antibacterial activity using disk diffusion growth
674 inhibition assays against putative coral pathogens, *Vibrio coralliilyticus* OfT6-21 and *V.*
675 *coralliilyticus* OfT7-21, *Leisingera* sp. McT4−56 and *Alteromonas* sp. McT4-15. To make
676 pathogen lawns, overnight liquid cultures of pathogens were grown by inoculating 2-3 mL of
677 seawater broth (4 g/L tryptone and 2 g/L yeast extract in 0.22 mm filtered seawater) with individual
678 colonies of each strain and shaking culture tubes at 150 RPM and 28 °C (Benchmark Incu-shaker
679 10LR). To coat seawater agar (seawater broth with 15 g/L agar) plates with a pathogen lawn, a
680 200 mL aliquot of liquid culture (OD600 =0.5) was added to each plate (150 mm x 15 mm) and
681 spread using sterile glass beads.

682 Coral partitions were tested by solubilizing partitions in MeOH to a concentration of 6.25
683 mg/mL and applying 4 µL aliquots to sterile paper disks (Whatman Grade 1) in triplicate (final
684 amount 25 µg/disk). A filter disc with 4 µL of nalidixic acid at 15.62 mg/mL (62.5 µg) was used as
685 a positive control. Negative controls were disks treated with MeOH only. Disks were given time to
686 dry completely and then carefully transferred with sterile forceps to seawater agar plates
687 containing freshly coated pathogen lawns. Disk diffusion plates were then incubated at 28 °C
688 overnight. After incubation, zones of inhibition (ZOI) were measured using digital calipers from
689 the edge of the paper disk to the edge of the zone of bacterial growth inhibition.

690

691 **Ethics**

692 The sample collection was covered by permit FKNMS-2019-160 to Valerie Paul.

693

694 **Data accessibility**

695 The metabolomics data utilized in this manuscript is available at gnps.ucsd.edu with MassIVE ID#
696 MSV000089633. The data acquired in negative mode is also available in this dataset, but is not

697 presented in this study due to the lack of high-confidence annotations. The code utilized in this
698 manuscript is available at https://github.com/Garg-Lab/Dry-Tortugas-Corals-Files.
699
700 **Competing interests**
701 We declare we have no competing interests.
702
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715
716
717

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Figure legends

Figure 1. Sample collection, data acquisition, and analyses. **(A)** Benthic map of the sample sites in relation to Dry Tortugas National Park. Colors denote the benthic habitats (partially transparent) overlaid on high-resolution bathymetry. Red and brown illustrates coral reef habitat (Florida unified reef map, Florida Fish and Wildlife Conservation Commission, 2016). A, B, C, D refer to the sites at which coral colonies were sampled. Coordinates for these sites are in Table S1. **(B)** Representative photographs of each coral species in this study (CNAT= *C. natans*, MMEA= *M. meandrites*, OFAV= *O. faveolata*, MCAV= *M. cavernosa*). **(C)** Untargeted metabolomics data were acquired, processed, and analyzed with a variety of methods. Metabolomics data available through public datasets (mined using MASST) and acquired on cultured algae was used to assign the biosynthetic source of annotated metabolite features. (D) Schematics of representative interactions between the coral holobiont members is shown. The host genotype, the microbiome composition, and the endosymbiont Symbiodiniaceae species as well as complex interplay of interactions between them can confer resilience to increased frequency and impact of coral diseases.

Figure 2. **Intra- and interspecies variation of metabolomics data. (A)** Principal component analysis of corals: *C. natans* (CNAT, red), *M. cavernosa* (MCAV, green), *M. meandrites* (MMEA, purple), and *O. faveolata* (OFAV, blue). The SCTLD susceptibility categorization is included in the key ('Highly', 'Moderately'). Axes are labeled with the corresponding variance explained by each principal component. A, B, C, D refer to the site from which the coral was sampled (Table S1). **(B)** Hierarchical clustering analysis reveals a separate cluster for all *M. meandrites* samples, while the other species are distributed across clades. Colored branches correspond to species as outlined in (A). The letter at the end of the sample name corresponds to the sampling site. The x-axis represents the distance between the samples/clades. **(C)** UpSet Plot showing distribution of detected metabolite features. The number above each bar represents the number of features in that intersection. "Set Size" denotes the total number of features detected in each coral species. The inset table includes number of features detected within each species, as well as the number of annotated features reported in this manuscript.

Figure 3. **Analyses of vitamin E family compounds. (A)** Network of annotated vitamin E family compounds with *m/z*_RT (*m/z*: mass to charge, RT: retention time in min). The * indicates these features have identical MS^2 spectra, but different retention times, representing isomeric species. **(B)** The detection pattern of annotated vitamin E family compounds in cultured endosymbiont extracts. The comprehensive list for annotated vitamin E family compounds is provided in Table S2, and MS^2 mirror plots supporting annotations of tocopherol(hydro)quinone are provided in Figure S4. The SCTLD susceptibility categorization is included in the key ('Highly', 'Moderately').

Figure 4. Annotation of and variation in detected acylcarnitines. **(A)** Chemical structure of docosatetraenoyl carnitine (C22:4). **(B, C, F-I)** A subset of the box plots of acylcarnitines differentially detected in the extracts of *M. meandrites*. Asterisks indicate significant differences as determined by a Kruskal Wallis test with Dunn's post-hoc test (adjusted $p<0.05$). Additional box plots are reported in Figure S4. **(D)** MS2LDA motif 185 used to aid annotations of features annotated as acylcarnitines. **(E)** The query submitted to MassQL to search for acylcarnitines. **(J)** Heat map of the features annotated as acylcarnitines that were determined as statistically differentiating by Kruskal Wallis test with Dunn's post-hoc test (adjusted $p<0.05$). The scale bar represents the log-transformed abundance. The putative annotation and *m/z*_RT are included for each feature (Table S2 and Figure S5). **(K)** The hierarchical clustering analysis based on the log-

1201 transformed abundances of the acylcarnitines. The SCTLD susceptibility categorization is
1202 included in the key (‘Highly’, ‘Moderately’).

1203 **Figure 5**. **Interspecies variation of diacylglyceryl-carboxyhydroxymethylcholine (DGCC)**
1204 **betaine lipids. (A-G)** Box plots of features annotated as DGCC(16:0) analogues. Annotations
1205 are provided in Figure S6, S7 and Table S2. Asterisks indicate significant differences between
1206 coral species as determined by Kruskal Wallis test with Dunn’s post-hoc test (adjusted $p<0.05$).
1207 **(H)** The detection pattern of DGCC analogues in the cultured zooxanthellae extracts. The SCTLD
1208 susceptibility categorization is included in the key (‘Highly’, ‘Moderately’).

1209 **Figure 6**. **Analyses of endosymbiont-derived pigments. (A)** Heat map showing the distribution
1210 of features annotated as pigments. The log-transformed abundance is reported. The *m/z*_RT and
1211 annotation are included. The * indicates these features have identical MS^2 spectra, but different
1212 retention times representing isomeric species **(B)** Hierarchical clustering analysis based on the
1213 log-transformed abundance of the annotated pigments shows no clustering by species nor site.
1214 The SCTLD susceptibility categorization is included in the key (‘Highly’, ‘Moderately’).

1215 **Figure 7. Analysis of BuOH partitions of crude extracts. (A)** Bioactivity of EtOAc and BuOH
1216 partitions of crude extracts determined using agar diffusion growth inhibition assay against coral
1217 pathogen *V. coralliilyticus* OfT6-21. Letters denote results from a Tukey HSD posthoc test
1218 following a significant one-way ANOVA (p<0.001). **(B)** Venn Diagram of features detected in
1219 whole coral extracts and BuOH partitions. 560 unique metabolite features were detected in BuOH
1220 partitions. UpSet Plot of all features detected in BuOH partitions, where the number above each
1221 bar represents the number of features in that intersection and “Set Size” denotes the total number
1222 of features detected within that coral species. (MMEA= *M. meandrites*, OFAV= *O. faveolata*,
1223 MCAV= *M. cavernosa*, CNAT= *C. natans*). The SCTLD susceptibility categorization is included in
1224 the key (‘Highly’, ‘Moderately’). **(C)** Chemical diversity of features detected exclusively in BuOH
1225 partitions, along with the distribution of these features among the coral species. Natural product
1226 pathways (probability >0.8) were determined using CANOPUS. **(D)** Chemical structures of
1227 putatively annotated metabolite features uniquely detected in BuOH partitions (annotations
1228 supported in Figures S8 and S9).
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