Apicomplexans predict thermal stress mortality in the Mediterranean coral *Paramuricea clavata*

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ABSTRACT

Paramuricea clavata is an azooxanthellate octocoral currently threatened by episodic mass mortality events related to global warming. The microbiome may play an active role in the thermal stress susceptibility of corals, potentially holding the answer as to why corals show differential sensitivity to heat-stress. The microbiome of *P. clavata* collected from around the Mediterranean was characterized right before experimental heat-stress using the 16S and 18S small subunits (SSU) of the rRNA gene to determine if it influences the thermal response of the holobiont. *P. clavata's* microeukaryotic community was significantly correlated with thermal stress sensitivity, while bacterial assemblages were not. Syndiniales dinoflagellates Group I Clade 1 were significantly enriched in thermally resistant corals, while the apicomplexan Corallicolida were significantly enriched in thermally susceptible corals. Our results show the importance of the microeukaryotic communities to understanding thermal stress response in corals and provides a useful tool to guide conservation efforts.

39 *Paramuricea clavata* is a slow-growing octocoral prevalent in the temperate Mediterranean
40 Sea (1). Gorgonians, such as *P. clavata*, are habitat-forming species of biodiversity-rich hard
41 bottom benthic communities where they provide complex habitat for numerous organisms
42 contributing significantly to the biodiversity of the region (2). *P. clavata's* slow growth rate, long
43 turn-over time, high post-settlement mortality, and low genetic connectivity puts it at high-risk of
44 extinction caused by natural and anthropogenic stressors (1). *P. clavata* populations have been
45 facing increased mortality due to episodic mass mortality events (MMEs) (3). MMEs, which were
46 once uncommon in temperate ecosystems such as the Mediterranean Sea, have been increasing
47 since the turn of the century, with most of these occurring in the Western Mediterranean (4). The
48 increase in the frequency, extension, and intensity of marine heatwaves driven by global warming
49 have been suggested to be the primary cause of the increase in MMEs in the Mediterranean. Thus,
50 MMEs are leading to the current biodiversity crisis in the region (5,6). *P. clavata* are heavily
51 affected by MMEs with mortality sometimes reaching 80% in affected populations and recovery
52 hindered by the recurrence of marine heatwaves. The loss of this habitat-forming species causes a
53 functional shift in Mediterranean hard-bottom communities leading to simplified habitats with
54 lower species diversity (7,8). To prevent the significant decline of *P. clavata* and other habitat
55 forming octocorals in the Mediterranean, the effect of thermal stress on the *P. clavata* holobiont
56 must be better understood.

57 Thermal stress typically causes death in anthozoans by disrupting metabolic pathways,
58 increasing physiological stress, decreasing host defense, causing energy constraints, and
59 promoting the growth of opportunistic pathogens (9–11). In *P. clavata,* exposure to higher
60 temperatures causes usually partial or full necrosis of colonies, where branches first change color
61 from red to grey before denudation of the skeleton (12). This contrasts with "bleaching" which

takes place in zooxanthellate corals and is when the algal symbiont leaves the coral host during periods of high heat-stress. *P. clavata* is more sensitive to elevated seawater temperatures than other gorgonians, marked by a significant loss in energy reserves, reduced feeding ability, and partial mortality after heat-stress (13). A complete characterization of the *P. clavata* microbiome before heat-stress can better elucidate the role of the microbiome to overall holobiont health and fitness in the face of warming ocean temperatures.

Octocoral-associated microorganisms are considered beneficial members of the coral holobiont contributing to nitrogen fixation, sulfur-cycling, antibiotic production, and host defense (14). *P. clavata* has a regionally stable bacteriome dominated (above ~90% relative abundance) by the common coral symbiont *Endozoicomonas,* a Gammaproteobacteria of the order Oceanospirillales (15,16). Notably, *P. clavata*'s bacterial community differs significantly from the microbiome of the water surrounding the organism and that of neighboring octocorals, such as *Eunicella* (17). Other bacteria commonly found within *P. clavata* include Alphaproteobacteria, Betaproteobacteria, and Actinobacteria (15). Despite the research into the bacterial community found in *P. clavata* throughout the Mediterranean Sea, there has been no research into the microeukaryotic community which constitutes a significant portion of the microbial diversity and can contribute significantly to holobiont fitness in a changing climate (18).

Microeukaryotes (protists and unicellular fungi) are important drivers of many of the Earth's ecosystems including being the main contributors to oceanic primary production and carbon mobilization in the microbial loop. They may influence the cycling of nutrients in the holobiont as well as play a role in host defense. The corallicolids, which are part of a lineage of parasitic alveolates known as the Apicomplexa are associated with 70% of corals globally (19). Apicomplexans also include such well-known human parasites like *Plasmodium*, the causative

85 agent of malaria, and *Toxoplasma*, a wide-spread mammal-infecting pathogen. While the
86 corallicolids, based on their distribution and presence in healthy individuals (20), seem to be
87 commensals in the coral microbiome, their evolutionary context suggests a more parasitic role for
88 this general anthozoan associate.

89 To better understand the microbiome of *P. clavata* and its ability to predict thermal stress
90 susceptibility we conducted a common garden heat-stress experiment using corals collected from
91 12 populations (148 total individuals) across the geographic range of *P. clavata* (Extended Data
92 Fig. 1). We used an 18S rRNA and 16S rRNA gene metabarcoding approach to get a holistic view
93 of the microbes which may confer thermal resiliency to *P. clavata.*

94 **RESULTS**

95 **The bacterial microbiome of *Paramuricea clavata***

96 For the 16S rRNA gene sequencing, 1 332 678 total reads were recovered after processing
97 in DADA2. Following filtering, a total of 4 540 Amplicon Sequence Variants (ASVs) across 92
98 samples were left for bacterial community analysis. The water and food microbiome samples were
99 taxonomically distinct from the coral microbiome samples in this study (Extended Data Fig. 2).
100 The population originally sampled from Sagres, Portugal exhibited a distinct microbiome from the
101 other *P. clavata* populations (Fig. 1) and was later determined to be a different species based on
102 genomic work (21)**.** It was hence removed from further analysis. Oceanospirillales, mostly
103 consisting of Endozoicomonas, constituted most of the bacterial community of *P. clavata* (Fig 1).
104 Other highly abundant bacterial orders include Alteromonadales, Rhodobacterales, and
105 Flavobacteriales. A high number of unidentified bacterial ASVs were also present in the dataset.
106 Subsequent BLAST analysis of these ASVs yielded no definitive taxonomic identification.

107 Pair-wise analysis of the bacterial communities between localities showed significant
108 differences in Shannon-Wiener alpha-diversity (Extended Data Fig. 3). Additionally, the beta-
109 diversity of the bacterial community between localities was significantly different as well
110 (ANOSIM; P = 0.001; Extended Data Fig. 4). Overall, the bacterial community of *P. clavata*
111 clustered closely based on geographic origin.

112 The dominance of Endozoicomonas (Proteobacteria; Oceanospirillales) in the bacterial
113 community of *P. clavata* masked any fine-scale differences in the bacterial community
114 composition across the range of thermal susceptibilities (Fig. 2). Analyzing the bacterial
115 community with Endozoicomonas removed, did not reveal any noticeable pattern in taxon
116 composition in relation to thermal stress susceptibility (Extended Data Fig. 5); however, an

117 ANCOMBC comparison between the super-resistant and hyper-sensitive thermal susceptibility
118 categories showed that Pseudomonadales and Francisellales were significantly more abundant in
119 hyper-sensitive corals (Fig. 3).
120
121 **The eukaryotic microbiome of *Paramuricea clavata***
122 For the 18S rRNA sequencing, 18 955 358 total reads were recovered, resulting in 3 181
123 ASVs across 138 samples following filtering. This community is dominated by 3 major clades of
124 Alveolates including: Apicomplexans, Ciliates, and Syndiniales (Fig. 1). One of the primary
125 microeukaryotic taxa present, Dino-Group-I (Dinoflagellata; Syndiniales), exhibited its highest
126 relative abundance in the more Eastern populations (Balun, Mana, & Lighthouse) (Fig. 1).
127 Corallicolids (Apicomplexa; Corallicolida) exhibited a high relative abundance across every
128 population sampled, excluding Lighthouse (Fig. 1). Additionally, three distinct genera of ciliates
129 (Ciliophora) were found across the surveyed geographic range of *P. clavata*, with *Licnophoria*
130 spp. being prevalent at Tascons, Lighthouse, and Altare (Fig. 1). *Hypotrichia* spp. were found
131 mainly in the Western populations (Tascons, Pota de Llop, La Vaca, Palazzu, Palazzinu, &
132 Gargallu) (Fig. 1). Lastly, *Suctoria* spp. were most prevalent within the *P. clavata* of the Medes
133 and Portofino localities (Fig. 1).
134 At the ASV-level, the locality-level microeukaryotic communities of *P. clavata* exhibited
135 higher Shannon-Wiener alpha-diversity compared to the bacterial communities (Extended Data
136 Fig. 3). Additionally, the microeukaryotic community only showed a significant difference in the
137 alpha-diversity of *P. clavata* between Croatia vs Medes. Furthermore, the beta-diversity between
138 localities was significantly different for the microeukaryotic community (ANOSIM; P = 0.001;
139 Extended Data Fig. 4).

140 Syndiniales were enriched in the super-resistant corals (LEfSe; P = 0.0016). Specifically,
141 Dino-Group I Clade 1 abundance in the holobiont was associated with the increased
142 thermotolerance in *P. clavata* and was enriched in the super-resistant individuals (LEfSe; P =
143 0.0027; Fig. 2). At the ASV level, ASV_20 and ASV_13 were the Dino-Group I Clade 1 ASVs
144 which were highly abundant in the resistant groups relative to the other Alveolates (Extended Data
145 Fig. 8). Concurrently, a high relative abundance of corallicolids within the holobiont was
146 associated with higher sensitivity to thermal stress (Fig. 2). LEfSe analysis revealed that the
147 Corallicolidae family was significantly enriched in the sensitive corals (LEfSe; P = 0.015). ASV_6,
148 within the COR3 clades of corallicolids, showed the highest abundance amongst of the alveolates
149 in the hyper-sensitive corals (Fig. 9). ANCOMBC analysis also confirmed these associations, with
150 corallicolids being significantly more abundant (averaging ~2.5 natural log fold higher abundance)
151 in the sensitive corals compared to the resistant ones, while Dino-Groups I and II showed an
152 opposite pattern (Fig. 3). On the other hand, ciliates showed a stable abundance throughout thermal
153 sensitivity groups, except for ASV_7 (*Ephelota mammillata*) which was highly abundant in the
154 sensitive and tolerant groupings (Extended Data Fig. 8). Alpha-diversity and beta-diversity
155 analysis of both the bacterial community and microeukaryotic community yielded no significance
156 differences between any of the thermal tolerance categories (Extended Data Figs. 3 & 4).
157
158 **Phylogenetic placement of the microeukaryotic microbiome**
159 Phylogenetic analysis of the most prevalent Alveolate ASVs found within *P. clavata*
160 revealed a diversity of clades associated with the Octocoral across the surveyed geographic range.
161 ASV_6, which was the most abundant corallicolid ASV and found in most samples, was most
162 abundant in the Scandola populations (Fig. 4). An evolutionary placement algorithm placed this

163 ASV as closely related to MN022452_Corallicolidae_COR3_sp. (99.22% Similarity), a sequence
164 recovered from the deep-sea Octocoral, *Callogorgia delta* in the Gulf of Mexico. A second clade
165 was identified that most likely belonged to the Corallicolidae COR2 group (Fig. 4). These ASVs
166 (ASV_16, ASV_19, ASV_22) have a low relative abundance within *P. clavata* but are found
167 across most samples (Fig. 4). This group is most closely related to
168 MN022429_Corallicolidae_COR2_sp. (95.84% Similarity to ASV_16), a sequence recovered
169 from the deep-sea Antipatharian *Leiopathes glaberrima*, also from the Gulf of Mexico. Lastly, a
170 third clade of prevalent corallicolids was identified within *P. clavata* that shows close relation to
171 JX943883_Corallicolidae_COR1_sp. (93.04% Similarity to ASV_105), a sequence found in the
172 stony coral *Porites astreoides,* from the Bahamas.

173 Examination of the Dinoflagellate ASVs revealed distinct clades present within *P. clavata*.
174 Dino-Group-I Clade 1 ASVs were most prevalent across the dataset with ASV_13 being the most
175 abundant across all localities except Scandola, where ASV_12 is most prevalent (Extended Data
176 Fig. 6). The closest blast results for these sequences were to uncultured microeukaryotes isolated
177 from intertidal mudflat sediments and ocean surface water from the China Sea (22,23) (100%
178 Similarity). One Symbiodiniaceae ASV (ASV_43) was identified in some of the populations
179 (Tascons, La Vaca, Palazzinu, Lighthouse, Altare, Mana & Balun) despite *P. clavata* being
180 azooxanthellate (Extended Data Fig. 6). The ASV from this study corresponds to Symbiodinium
181 Temperate Clade A which has been identified in Mediterranean corals before (24). It's closest blast
182 result from NCBI was to a *Symbiodinium sp.* Temperate Clade A sequence (99.21% Similarity)
183 recovered from the stony coral *Mussismilia hispida* (25).

184 The ciliate ASVs within the dataset revealed three primary clades found variably abundant
185 across the surveyed distribution of *P. clavata*. ASVs corresponding to *Holostichia diademata*

186 (Order: Hypotrichia) (ASV_8) and *Ephelota mammillata* (Order: Suctoria) (ASV_7) were the most
187 abundant and prevalent across the dataset overall (Extended Data Fig. 7). Interestingly, BLAST
188 results for ASV_8, shows 100% similarity to *Holosticha pullaster* (Order: Hypotrichia) (100%
189 Similarity) isolated from a freshwater pond in the USA (26). ASV_7 showed high similarity to *E.*
190 *mammillata* recovered from *Tubularia* hydroids (98.51% Similarity) (27). *Licnophora*
191 *macfarlandi* (Order: Licnophoria), was found across most populations, with its highest abundance
192 being in Mana, Croatia (Extended Data Fig. 7). Blast results show that these sequences were highly
193 similar to *L. macfarlandi* (92.66% Similarity to ASV_3) recovered from the fluid in the respiratory
194 trees of the giant California sea cucumber *Parastichopus californicus* (28).
195
196 **Co-occurrence between members of the microbiome**
197 A microbial co-occurrence network was constructed using SPARCC to analyze the positive
198 correlations between the major microbial families of the *P. clavata* holobiont. Corallicolidae &
199 Dino-Group I Clade 1 had the strongest significant co-association between all the major taxa (Fig.
200 5). This co-association is of note when you consider these two group's antagonistic relative
201 abundance patterns across the thermal susceptibility groups (Fig. 2). The three ciliate families
202 showed consistent associations with the corallicolids and Dino-Group I Clade 1. Endozoicomonas,
203 Pseudoalteromonadaceae, Vibrionaceaee, and Flavobacteriaceae were the primary bacterial
204 families that showed significant co-occurrence with the different microeukaryotic members of the
205 *P. clavata* holobiont (Fig. 5).
206
207 **DISCUSSION**

208 The bacterial community of *P. clavata* showed very little differences in its taxonomic
209 make-up from location to location (Fig. 1), which is consistent with previous work into the
210 bacterial component of the *P. clavata* holobiont (16,29). The differences in beta-diversity and
211 alpha-diversity between the different localities where *P. clavata* was sampled can be contributed
212 to the vast diversity of Endozoicomonas found across the surveyed distribution. The prevalence of
213 unidentified bacterial ASVs in the dataset points to the underrepresentation of octocoral-associated
214 bacteria in the SILVA reference database. These results exemplify the need to identify more
215 bacteria associated with a healthy symbiotic state in coral holobionts.

216 The microeukaryotic community of *P. clavata* is dominated by three major groups of
217 alveolates: Apicomplexa (mostly corallicolids), Dinoflagellata (mostly Syndiniales), and
218 Ciliophora. This community shows more variability in its taxonomic make-up from location to
219 location, while also showing significant differences in beta-diversity (Fig. 1 & Extended Data Fig.
220 4). Each of the alveolates found in *P. clavata* showed locality-specific differences, highlighting
221 the specificity and potential co-adaptations of these associations in coral holobionts (Fig. 4,
222 Extended Data Figs. 6 & 7). Corallicolids are general anthozoan associates found world-wide,
223 even at depths as deep as 1400 m, and in some cases can be as abundant as Symbiodiniaceae
224 (30,31). They tend to be the most abundant and prevalent microeukaryotic coral symbiont in
225 azooxanthellate corals. The ones recovered from this study showed close relation to those found
226 within deep-sea cnidarians, including octocorals and antipatharians, of the Gulf of Mexico from
227 Vohsen *et al.* (32) (Fig. 4). A third clade of corallicolids were closely related to one recovered
228 from the shallow water Caribbean coral *Porites astreoides* (33). This diversity of corallicolids
229 present within *P. clavata,* alone, hints at the potential global diversity and ubiquity of corallicolids
230 in anthozoan hosts.

231 Syndiniales, which have been found in high prevalence in reef environments before, also
232 constituted a major portion of the eukaryotic microbiome. They are an order of parasites found at
233 the base of the dinoflagellates which can infect a variety of marine macro- and microscopic
234 organisms (34). Syndiniales from Dino-Group I are one of the main microeukaryotic taxa found
235 in the hard coral *Pocillopora damicornis* (35), however this group is also ubiquitous in global
236 plankton communities (36). Additionally, Dino-Group 1 Syndiniales with identical 18S rRNA V4
237 sequences may associate with different hosts, suggesting a generalist host specificity for this clade
238 or the need for a different marker gene to decipher its true diversity (37). Syndiniales have the
239 potential to disrupt nutrient cycling, and thus would likely play a role in (de)stabilizing symbiotic
240 relationships, which rely on nutrient dynamics, in a tight-knit symbiotic system such as a coral
241 holobiont. Their differential abundance within *P. clavata* across different thermal susceptibility
242 categories highlights the necessity of studying the role of parasitism in the nutritional dynamics of
243 marine holobionts, especially in the face of stressors. Hudson et al. 2006 noted that a healthy
244 system may be one that is rich in parasitic species due to the impact of parasite-mediated effects
245 on population dynamics, interspecific competition, and energy flow (38). Applying this
246 perspective to coral holobionts may help us better understand the higher thermal tolerance of *P.*
247 *clavata* rich in Syndiniales. The high abundance of these parasites within the *P. clavata* holobiont
248 may contribute to a healthy and resilient system by influencing the microbial community dynamics
249 when confronted with heat-stress.

250 Within the ciliate lineages recovered from *P. clavata*, three genera (*Licnophora*,
251 *Holosticha*, & *Ephelota*) showed variable abundances across the surveyed distribution of the coral
252 host. *L. macfarlandi*, has been found as an endosymbiont in the respiratory trees of holothuroids,
253 but also in association with corals, likely as a secondary invader hunting other microbes (39,40).

254 It is a noted predator of protozoans and is speculated to be attracted to other ciliate species found
255 at the interface of coral lesions (40). *L. macfarlandi* within *P. damicornis* also exhibited geographic
256 variability in abundance (35), which may depend upon the presence of other ciliates on which it
257 preys. Another ciliate, *H. diademata,* has also been found within corals often crawling slowly on
258 the coral surface (40). It is a noted bacterivore so it may feed upon the bacteria of the coral, exerting
259 top-down control upon that portion of the microbiome, and therefore influencing the cycling of
260 certain nutrients in the holobiont. The presence of bacterivores may increase microbial activity
261 and turnover within the ecosystem (41). While the previous two ciliates are predominately
262 associated with diseased corals (42), the third ciliate genera, *Ephelota,* has never previously been
263 described in corals, however Suctorian ciliates are typically commensal and have previously been
264 recovered as epibionts of crustaceans, mollusks, fishes, and even other protists (43). Thus, it may
265 be using the stability and structure provided by *P. clavata* to stabilize itself in the water column
266 and capture prey more readily.

267 This study identified the key microbes associated with *P. clavata,* an important habitat-
268 forming species impacted by MMEs, and provides evidence of the microbiome's potential role in
269 the thermal susceptibility of the host. One of the bacterial taxa found to be associated with
270 thermally resilient coral was Pseudomonadales, which may be beneficial to a healthy coral
271 holobiont as it can prevent the growth of harmful Flavobacteria which may lead to disease (44).
272 However, in *P. clavata*, the overwhelming abundance of Endozoicomonas didn't allow for any
273 other fine scale differences in bacterial composition to be detected. On the other hand, the
274 microeukaryotic community has distinct taxonomic composition associated with different degrees
275 of thermal tolerance. Dino-Group I Clade 1 was significantly enriched in thermally tolerant corals,
276 while corallicolids were significantly enriched in thermally sensitive corals. How the relative

277 abundance of these apicomplexans confers thermal susceptibility to the coral host is not yet
278 understood. There's the possibility that the symbiosis between corallicolids and their coral host
279 shifts from an apparent commensalism towards parasitism as added stressors, such as heat,
280 breakdown the relationship. The context-dependency of this relationship can explain the
281 abundance of corallicolids in apparently healthy coral. The coral host may have evolved resistance
282 to corallicolids at a significant fitness cost leading to a state of commensalism without the presence
283 of addition stressors; however, heat-stress may cause the corallicolid-tolerant corals to exhibit
284 higher mortality as the apicomplexans shift towards parasitism, similar to the hypothesis proposed
285 by Miller et al. 2006 (45). This potential interaction and the widespread distribution of the
286 corallicolids would have significant implications when interpreting threats to corals in the context
287 of warming ocean temperatures.

288 Showing strong co-association with the corallicolids were the Syndiniales, specifically
289 those belonging to Dino-Group I Clade 1 (Fig. 5). These parasites are known to infect a variety of
290 multicellular and microbial hosts and have previously been recovered from coral reef sediments,
291 plankton, and corals (46). Members include microeukaryotic parasites such as *Duboscquella sp.*
292 which is known to parasitize ciliates (47). This opens the possibility that they may be infecting
293 either the coral host, it's microeukaryotic members, or both. While significant positive co-
294 association between Dino-Group I and the ciliates recovered from *P. clavata* was apparent in the
295 network analysis, the association between Dino-Group I and the corallicolids was most prevalent
296 (Fig. 5). While this can simply be the result of fluctuations in two of the most highly abundant
297 microeukaryotes found in the dataset, it may also represent an interesting case of parasitism of a
298 coral endosymbiont by another member of the microeukaryotic community. Its association with
299 the corallicolids hints at a possible positive role of this parasite preventing the overabundance of

corallicolid growth in the coral holobiont which may be associated with decreased thermal tolerance. Syndiniales have been proposed to have the capacity to control the sizes of their host populations since a singular infection can produce hundreds of dinospores to further infect new hosts (48). The vast diversity of known microeukaryotic hosts of syndiniales combined with the larger cell size of corallicolids (~12 um in length (19) supports the possibility of this relationship. Possible explanations for this interaction could be top-down control of the corallicolid population by the Syndiniales, niche competition, easier co-infection of an already infected host, or excess dinospore production by Syndiniales. In either case, the relationship between Dino-Group I, the corallicolids and the coral host needs to be investigated further to decipher the ecological relevance of this potentially pivotal interaction within the coral holobiont.

We have shown here that a deeper characterization of the often-overlooked microeukaryotic portion of the coral microbiome can have major implications of coral health in a warming ocean. Along with other genetic, environmental, and physiological mechanisms the microbiome should be considered a vital component when assessing coral thermal response. Future research should focus on identify the mechanisms by which these microbes confer thermal resiliency to individual corals and how this knowledge can best be used to manage fragile coral ecosystems.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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ONLINE METHODS

Sample Collection

Branch fragments (30 colonies per population) of healthy adult *P. clavata* were collected from four localities (11 discrete populations) encompassing much of the known Mediterranean distribution of the octocoral. 10-17 individuals per population were included in microbiome analyses, depending on their availability. From Croatia, *P. clavata* populations from Balun (Lat, Long; 43.803889, 15.255000) and Mana (43.800273, 15.266389) were sampled. Three populations were sampled from Portofino (Liguria, Italy): Altare (44.308918, 9.178645), Indiano (44.312476, 9.166889), and Lighthouse (44.298650, 9.219014). Three populations were sampled from Scandola (Corsica, France): Palazzinu (42.379854, 8.549999), Gargallu (42.371839, 8.534394), and Palazzu (42.379873, 8.546163). Three populations were sampled from Medes Islands (Catalonia, Spain): La Vaca (42.048047, 3.226322), Pota de Llop (42.0497 ,

344 3.2254), and Tascons (42.042189, 3.2269). Lastly, a population thought to be *P. clavata* was
345 sampled from an Atlantic locality from Sagres, Portugal (37.012129, -8.924541).
346
347 **Thermal Stress Experiment**
348 All branch fragments were transported to the Institut de Ciències del Mar (CSIC) in
349 Barcelona, Catalonia, Spain where they were housed in aquaria containing locally sourced
350 seawater. Colony fragments were distributed in different tanks, according to their sampling sites
351 and the two temperature treatments. Thermal treatments consisted of one control at which water
352 temperature was maintained at 18ºC, and another heated treatment at which water temperature was
353 maintained at 25ºC. The gorgonians were fed with copepods (*Cyclops* spp.) until the end of the
354 experiment. Following 21 days of incubation, the response of the coral fragment was assessed by
355 measuring the percentage of tissue necrosis. Thermal response was categorized as follows: Super-
356 resistant (0% necrosis), Resistant (< 25% necrosis), Tolerant (25-75% necrosis), Sensitive (>75%
357 necrosis), and Hyper-sensitive (100% necrosis). Extended Data Table 1 shows the thermal
358 resiliency categories for each coral sample at the end of the experiment. For each sample, 0.5 cm
359 length fragments were collected into a 2 mL cryovial containing 96% EtOH and stored at -80ºC.
360
361 **DNA Extraction and rRNA Gene Metabarcoding**
362 *P. clavata* samples were thawed at room temperature and then processed using a modified
363 DNeasy Mini Kit (QIAGEN) protocol. DNA concentration from purified samples was measured
364 using Qubit fluorometer. Concentrations that were higher than 1 ng/µl were considered acceptable
365 for PCR. The V4 region of the prokaryotic 16S rRNA gene was amplified according to the Earth
366 Microbiome Project protocol (49) and the V4 region of the eukaryotic 18S rRNA gene was

367 amplified using a nested PCR approach validated in del Campo et al. 2019 (50). Briefly, for the
368 18S rRNA metabarcoding, the samples were first amplified with UNonMet-PCR primers which
369 reduce the metazoan signal (18S-EUK581-F 5'-GTGCCAGCAGCCGCG-3′, 18S-EUK1134-R
370 5'-TTTAAGTTTCAGCCTTGCG-3′), then reamplified with primers from Comeau et al. 2011
371 (51). The PCR products were cleaned using QIAquick PCR Purification Kit (Qiagen). DNA
372 concentrations from purified PCR samples were checked using Qubit fluorometer before being
373 sent to the Integrated Microbiome Resource facility at the Centre for Comparative Genomics and
374 Evolutionary Bioinformatics at Dalhousie University for further 18S rRNA amplification and
375 sequencing.
376
377 **Microbiome Analysis**
378 Raw sequencing data for this project has been deposited on NCBI (XXXXXX). Primers
379 were removed from reads using Cutadapt v3.1 (52). The trimmed reads were then processed in R
380 using DADA2 (53). For the 18S rRNA gene amplicons, forward reads were truncated at 230 bp
381 and reverse reads were truncated at 195 bp. Reads were then denoised and joined into amplicon
382 sequence variants (ASVs). Chimeras were removed using the 'removeBimeraDenovo' command
383 with the "consensus" option. Taxonomy was assigned to ASVs using PR2 v4.13.0 (54). For the
384 16S rRNA gene amplicons, forward reads were truncated at 250 bp and reverse reads at 210 bp.
385 Truncated reads were then denoised and joined into ASVs before assigning taxonomy using
386 SILVA v138 (55). Sequence tables, taxonomy tables, and metadata for both the 16S and 18S
387 amplicon datasets were uploaded into a Phyloseq object in R for further filtering and analysis (56).
388 For the 16S rRNA gene amplicons, ASVs corresponding to Chloroplast and Mitochondria were
389 removed. One sample was also removed for having under 250 reads. Additionally, in each sample

390 ASVs that represent 1% or less of the reads were discarded. Following this filtering 4 546 ASVs
391 and 92 samples remained. The 18S amplicon were subjected to the same filtering parameters
392 except ASVs corresponding to Metazoa and Embryophyceae were also removed from the dataset,
393 resulting in 3 181 ASVs across 138 samples. Bubble plots, alpha-diversity, and beta-diversity
394 figures were constructed using ggplot2 and tidyverse packages (57). Statistical analyses were
395 conducted using the microbiomeMarker package and vegan (58,59). Analysis of similarities
396 (ANOSIM) within vegan was used to test for differences in beta-diversity between groups using
397 999 permutations. Linear Discriminant Analysis Effect Size (LEfSe) and Analysis of
398 Compositions of Microbiomes with Bias Correction (ANCOMBC) were used to find significantly
399 enriched and differentially abundant taxa between treatment groups.

400 In order to place the corallicolid ASVs in a phylogenetic context, a multiple sequence
401 alignment (MSA) was created using publicly available full-length 18S rRNA sequences of
402 corallicolids and the program MAFFT. Abundant corallicolid ASVs (more than 50% of
403 corallicolid signal in at least one sample) were then extracted from the dataset. Hmmalign was
404 then used to map these ASVs to the full length 18S rRNA corallicolid MSA (60). A maximum
405 likelihood phylogenentic tree was constructed with RAxML using this MSA (GTRCAT
406 substitution model, N = 1 000) (61). The resulting tree was modified for publication using ggtree
407 (62). For the dinoflagellate and ciliate trees, a similar approach was taken, except no publicly
408 available sequences were used in their construction and alignment.

409 To assess cross-domain positive-correlation in the occurrence patterns of ASVs, the 16S
410 and 18S amplicon tables were combined for samples present in both datasets. After setting a filter
411 prevalence threshold of 0.001, correlation patterns were inferred for the combined dataset using
412 SPARCC which considers the compositional nature of metabarcoding data (63). A network was

413 then constructed using SpiecEasi with a p-value threshold of 0.05 (ref. 30). The top families
414 exhibiting positive co-occurrence relationships were plotted in a chordplot using the MicroEco
415 package (65). All bioinformatic scripts used in this study can be found at
416 https://github.com/Abonacolta/Bonacolta_2022_Pcla.

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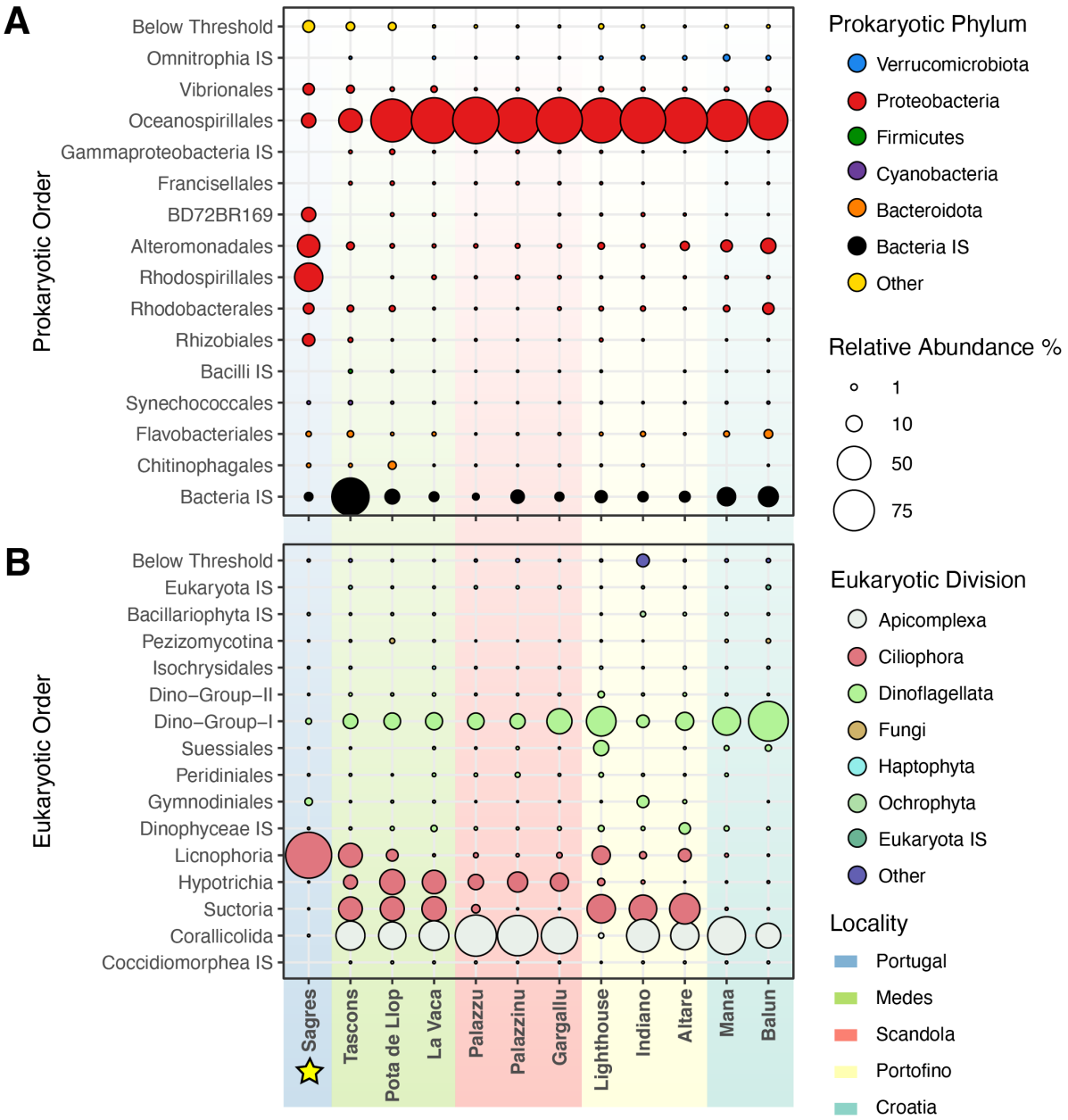
599

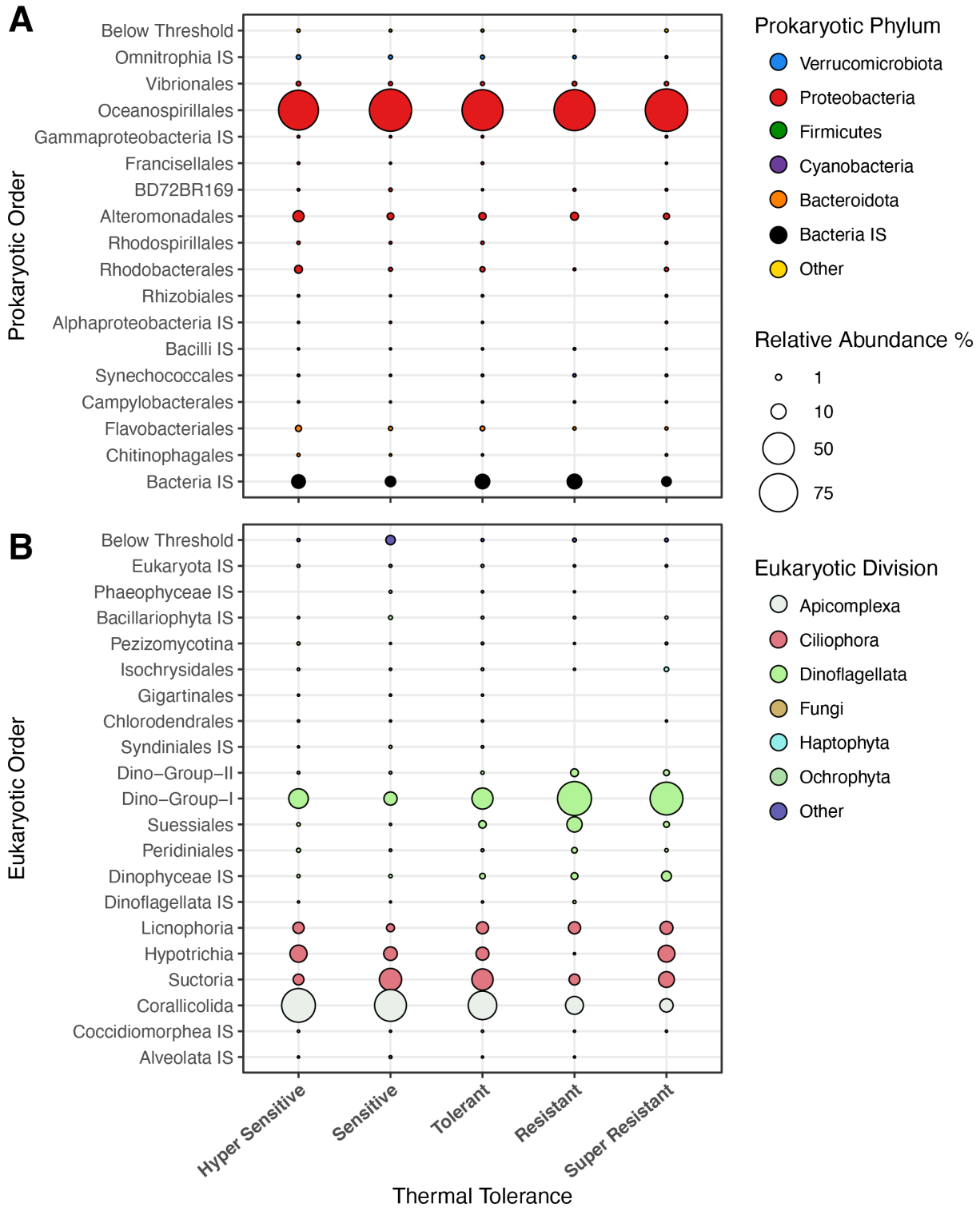
600 **Figure Legends**



601

602 **Figure 1.** Relative abundance of microbial orders within *P. clavata* across its geographic
603 distribution according to 16S rRNA gene amplicons (A) and 18S rRNA gene amplicons (B)
604 metabarcoding. *P. clavata* populations are listed from west to east, with major localities color
605 coded. Sagres is denoted with a star as this population was excluded from further analysis since it
606 is a different species. Microbial taxa are ordered taxonomically. Only those taxa with a median

607 relative abundance above 1% are shown, the rest are listed as “other”. IS is short for Incertae sedis,
608 meaning there is uncertainty in the taxonomic position within the broader taxonomic group.
609 Relative abundance bubbles correspond to within domain (Prokaryotic or Eukaryotic) relative
610 abundance. The Sagres population was removed from further analysis after being determined to
611 be a different species.



612

613 **Figure 2.** Relative abundance of microbial orders within *P. clavata* across thermal susceptibility

614 categories according to 16S rRNA gene amplicons (A) and 18S rRNA gene amplicons (B)

metabarcoding. The Sagres population was not included in this analysis (see Methods section). Hyper Sensitive (n_{bac} = 29; n_{euk} = 39) corals were those which showed 100% necrosis during the heat experiment. Sensitive (n_{bac} = 3; n_{euk} = 16) corals were those that showed < 75% necrosis. Tolerant (n_{bac} = 42; n_{euk} = 61) corals were those that showed 25-75% necrosis. Resistant (n_{bac} = 3; n_{euk} = 7) corals showed < 25% necrosis at the end of heat-stress. Super Resistant (n_{bac} = 4; n_{euk} = 5) corals showed 0% necrosis at the end of heat-stress. Microbial taxa are ordered taxonomically. Only those taxa with a median relative abundance above 1% are shown, the rest are listed as "other". IS is short for Incertae sedis, meaning there is uncertainty in the taxonomic position within the broader taxonomic group. Relative abundance bubbles correspond to within domain relative abundance.

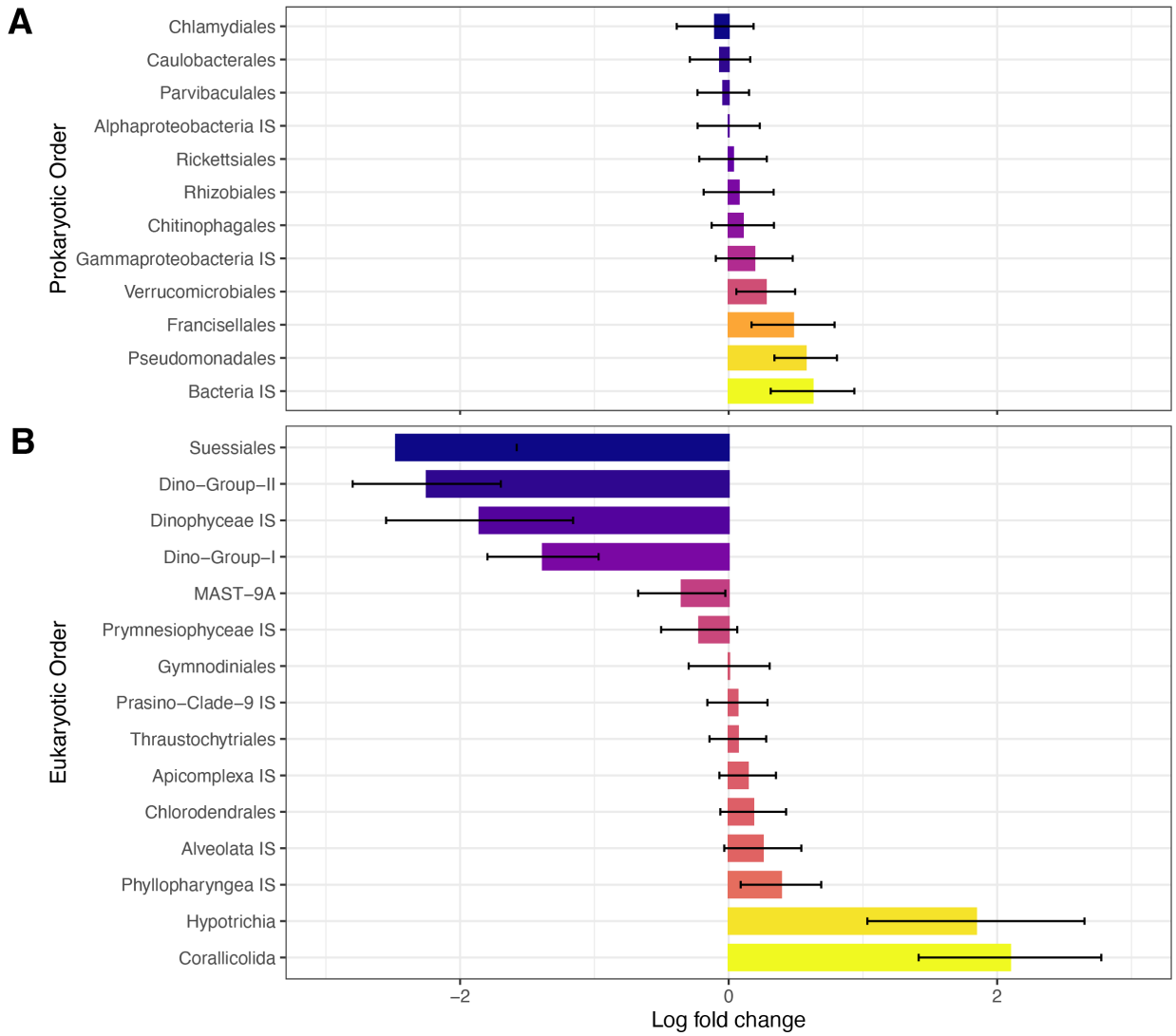


Figure 3. Significant differentially abundant microbial taxa between sensitive (hyper sensitive + sensitive; $n_{bac} = 32$; $n_{euk} = 55$) vs resistant (super resistant + resistant; $n_{bac} = 7$; $n_{euk} = 12$) samples according to ANCOMBC analysis. Natural log fold differences in abundance for each significant taxa are shown with SD. IS is short for Incertae sedis, meaning there is uncertainty in the taxonomic position within the broader taxonomic group. Panel A shows the bacterial taxa, while panel B shows the eukaryotic taxa.

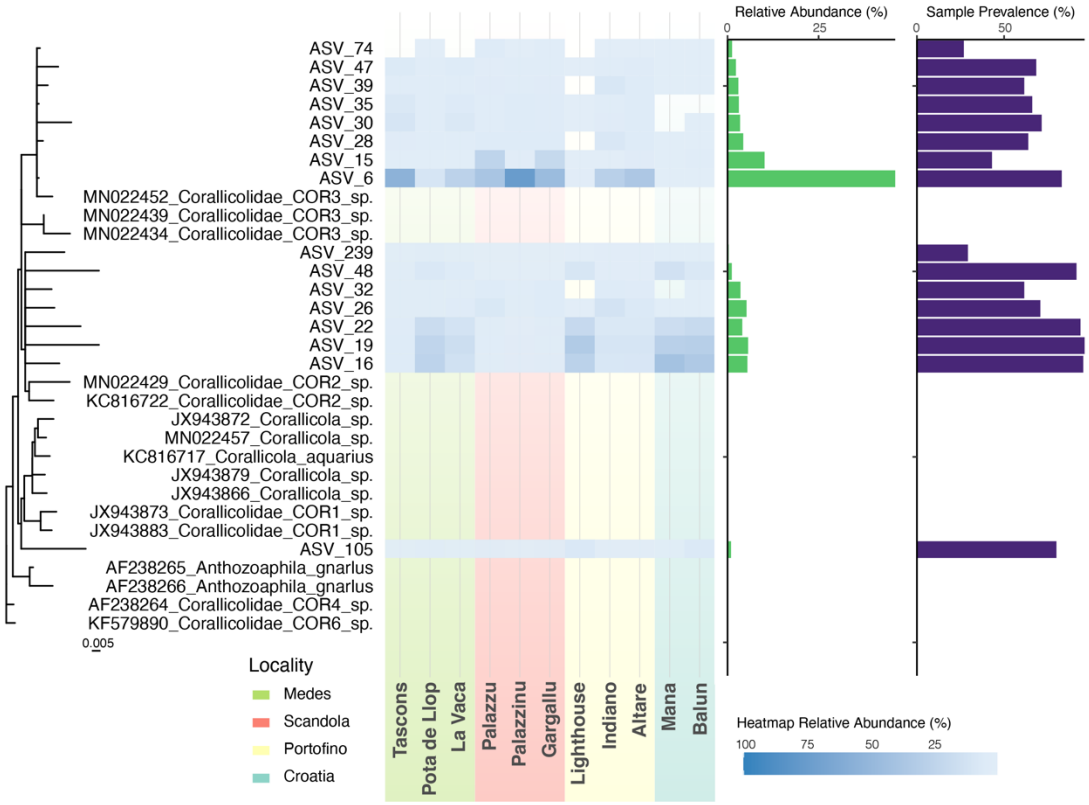


Figure 4. RAxML maximum-likelihood tree of Corallicolids with the primary Corallicolid ASVs recovered from *P. clavata* placed within the tree structure using EPA. Relative abundance of each ASV at each respective population is shown in the heatmap. Populations are ordered from west to east, with major localities color coded. Relative abundance (green) and sample prevalence (purple) of the ASV across the entire dataset is shown in the barplots to the right.

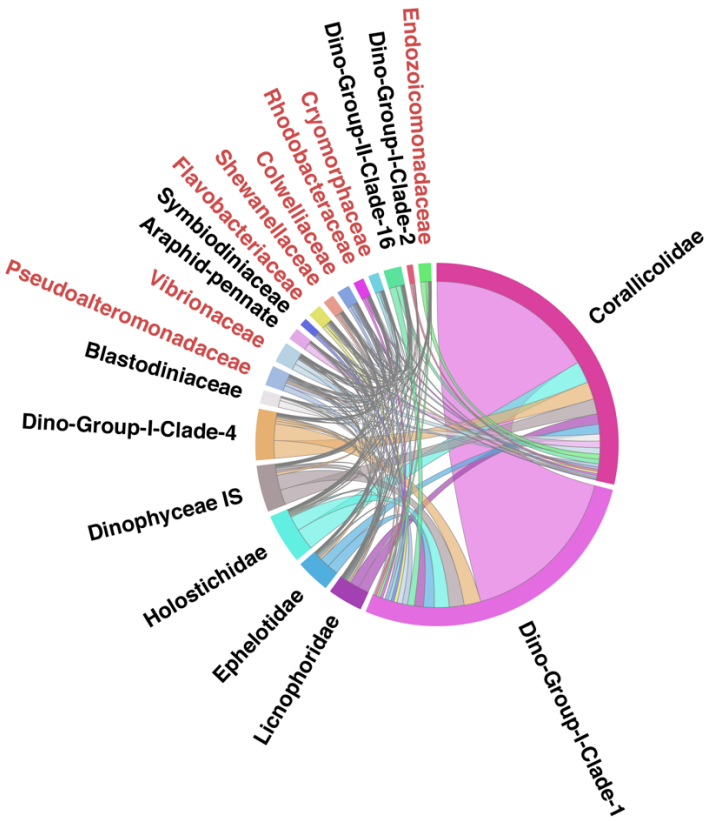


Figure 5. Chord plot of significant positive co-association patterns between the top microbial families across both the 16S and 18S rRNA gene amplicon datasets for *P. clavata*. Interactions were constructed using SPARCC and SpiecEasi with a p-value threshold of 0.05 for significance. Self-interactions have been removed. Eukaryotic families are shown in black text, while Prokaryotic families are shown in red text. IS is short for Incertae sedis, meaning there is uncertainty in the taxonomic position within the broader taxonomic group.