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1 **Inter- and intraspecific diversity in bacterial communities associated with two crustose**  
2 **coralline algae from the NW Mediterranean Sea**

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16

17 **Abstract**

18 Crustose coralline algae (CCAs) are key components and primary builders of coralligenous reefs,  
19 which are among the most biodiverse marine habitats in the Mediterranean Sea. Beyond their  
20 structural role, CCAs play a crucial role in promoting larval settlement in a variety of invertebrate  
21 species, including corals. In addition, CCAs form complex holobionts through association with  
22 symbiotic microorganisms that can modulate their ability to induce larval settlement and provide  
23 information on their health status. Despite their ecological significance and potential for  
24 biomonitoring, the microbial communities associated with Mediterranean CCA holobionts remain  
25 largely understudied. Using electron microscopy and barcoding techniques, we identified

26 *Lithophyllum stictiforme* complex and *Macroblastum dendrospermum*, two widespread CCAs  
27 from the Western Mediterranean Sea, living in sympatry with gorgonians. The 16S rRNA gene  
28 amplicon sequencing revealed that these CCAs host distinct and diverse bacterial communities,  
29 even among thalli of the same species, with closer thalli showing more similar bacterial  
30 assemblages. Our findings suggest that the primary factors influencing the bacterial communities  
31 include the host genotype, local environmental conditions at the individual thallus scale, and  
32 possible horizontal transfer between adjacent thalli. The predominance of bacterial sequences  
33 affiliated to the *Blastopirellula* genus and *Pir4\_lineage* in phylum Planctomycetes across all CCA  
34 individuals examined is noteworthy. These bacterial groups have been hypothesized to promote  
35 coral larvae settlement in tropical regions, raising the possibility of a similar facilitative interaction  
36 between CCAs and Mediterranean coral larvae.

37

38 **Keywords:** marine holobiont, microbiome, *Lithophyllum stictiforme*, *Macroblastum*  
39 *dendrospermum*, marine monitoring, coralligenous habitat

40

## 41 **1. Introduction**

42 Crustose coralline algae (CCAs) are a group of rhodophytes characteristic of coastal and  
43 mesophotic seascapes (Kahng et al., 2010; Castellán et al., 2022; Schubert et al., 2020). In the  
44 Mediterranean, they are key structural components of coralligenous concretions, which, due to  
45 their wide spatial and bathymetric extent (up to 2,700 km<sup>2</sup> in area and from 20 to 130 m depth;  
46 Martín et al., 2014, Iborra et al., 2022), constitute one of the most representative and biodiverse  
47 benthic habitat in the region (Ballesteros, 2006; Bracchi et al., 2022). Mediterranean CCAs also  
48 form a highly biodiverse group, potentially harboring significant cryptic diversity at both species

49 and genus level (Pezzolesi et al., 2019; Rindi et al., 2019). Through their calcareous three-  
50 dimensional thalli, CCAs serve as ecosystem engineers fostering high species richness (Asnaghi  
51 et al., 2015; Pezzolesi et al., 2017; Piazzini et al., 2022). Like their tropical counterparts,  
52 Mediterranean CCAs are increasingly affected by climate change and pathogenic diseases (Martin  
53 & Gattuso, 2009; Short et al., 2015; Quéré et al., 2019; Cornwall et al., 2022; Vitelletti et al.,  
54 2023), with widespread documented cases of mortality (Hereu & Kersting, 2016). As a whole, this  
55 context underscores the urgent need for targeted research on CCAs to better assess coralligenous  
56 biodiversity and health status.

57         Although the taxonomy, biology, and distribution of CCAs have been studied for a limited  
58 number of species, one of the least explored aspects is their associated microbiome (Rindi et al.,  
59 2019). To date, only two studies have addressed this topic focusing on *Neogoniolithon brassica-*  
60 *florida* (Quéré et al., 2019; Gefen-Treves et al., 2021). These studies provided crucial insights into  
61 CCA-bacteria associations and emphasized the need for further investigations into the functioning  
62 of these holobionts. There is growing evidence that CCAs harbor species-specific microbiomes  
63 (Barott et al., 2011; Cavalcanti et al., 2014; Sneed et al., 2015), with the associated bacteria playing  
64 a key role in the morphological development of the algae and exhibiting antifouling properties  
65 against epibionts (Singh & Reddy, 2016; Gómez-Lemos & Diaz-Pulido, 2017; Singh et al., 2017).  
66 For instance, bacteria can facilitate the settlement of *Ulva* spores (Joint et al., 2002), produce  
67 bioactive compounds that promote algal morphogenesis (Matsuo et al., 2005), and even form  
68 biofilms on CCAs that inhibit colonization by epiphytes, thereby reducing competition for light  
69 (Gómez-Lemos & Diaz-Pulido, 2017). The microbial community associated with algae may also  
70 serve as an indicator of algal and habitat health status (Glasl et al., 2017; Quéré et al., 2019). For  
71 instance, Zozaya-Valdes and colleagues (2015) identified a pathogen affecting the alga *Delisea*

72 *pulchra*, providing valuable information for predicting the spread of disease. Studies on  
73 Mediterranean CCA holobionts are crucial for establishing a foundation for future biomonitoring  
74 and conservation measures.

75 CCAAs are also known to facilitate the recruitment process of several invertebrate species  
76 (Lau et al., 2005; O’Leary et al., 2017; Seabra et al., 2019), promoting the settlement and  
77 metamorphosis of tropical coral larvae through chemical cues (Gómez-Lemos et al., 2018; Siboni  
78 et al., 2020; Jorissen et al., 2021). In the Mediterranean, field observations and an experimental  
79 study also showed gorgonian-CCA facilitative interactions (Toma et al., 2022; Zelli et al 2020).  
80 Gorgonian species, which are priority for conservation, are highly threatened by climate change  
81 and local impacts (Iborra et al., 2022; Tignat-Perrier et al., 2022; Chatzimentor et al., 2023). In  
82 recent decades, mass mortalities related to marine heat waves have severely affected shallow  
83 gorgonian populations (10-40 m, Garrabou et al., 2022; Bramanti et al., 2023). Efforts are currently  
84 underway to reintroduce these species to their natural habitat (e.g., Bramanti et al., 2007; Linares  
85 et al., 2008; Montseny et al., 2019; Casoli et al., 2022). Understanding how CCA holobionts  
86 interact with gorgonians (e.g., fostering larval settlement) could provide valuable insights for  
87 restoration strategies.

88 In this study, we analyzed the diversity of the bacterial communities associated with two  
89 widespread CCAs from the Western Mediterranean, *Lithophyllum stictiforme* and *Macroblastum*  
90 *dendrospermum*, both of which are either colonized by or in close proximity to gorgonians. To  
91 ensure consistent sampling, algae were collected in a restricted geographical area (approximately  
92 30 m<sup>2</sup>) to maximize the likelihood of obtaining thalli from the same species in similar  
93 environmental conditions. Previous research, in fact, has shown that bacterial communities  
94 associated with macroalgae, including CCAs, can vary considerably between individuals of the

95 same species due to diverse environmental conditions and exogenous factors (Staufenberger et al.  
96 2008; Burke et al. 2011; Barott et al. 2011; Miranda et al. 2013; Gefen-Treves et al., 2021).

97         Given the high cryptic diversity of CCAs (Rindi et al., 2019), which often makes  
98 identification based on morphological characters alone difficult, we used a combination of  
99 morphological traits and molecular techniques to identify these CCA species. To investigate  
100 species-specific bacterial associations, we characterized the diversity of bacteria associated with  
101 CCAs at different taxonomic levels using DNA metabarcoding. We further investigated both the  
102 common and distinct components of bacteria diversity, as well as the differential enrichment of  
103 bacterial taxa in CCA holobionts. These analyses were performed both between individuals of the  
104 two algal species and among individuals within the same CCA taxonomic group.

## 105         **2. Material and Methods**

106

### 107         ***2.1 Samples collection and preparation***

108 Thalli of CCAs (ca. 5 cm wide and 5-10 cm long) were collected in December 2022 at the Canadels  
109 site in Banyuls-sur-Mer, France (42°26.900' N, 3°10.347' E). The sampling area hosts populations  
110 of two octocoral species: the white gorgonian (*Eunicella singularis*) and the Mediterranean red  
111 coral (*Corallium rubrum*). The thalli of each CCA species were collected at three neighboring sites  
112 (*L. stictiforme*: ST25, ST27, ST7; *M. dendrospermum*: ST12, ST13, ST17), in three replicates per  
113 site, (n = 18). Samples were collected using hammer and chisel at 25 m depth by SCUBA diving.  
114 The sampling area covered approximately 30 m<sup>2</sup> and the distance between the samples and the  
115 octocoral colonies varied from 30 cm and 2 m.

116         In the laboratory, the epiphytes were carefully removed from the CCA surfaces with sterile  
117 forceps. After cleaning, each thallus was divided into fragments. One half was immediately frozen

118 at -80°C for DNA extraction, the other half was air-dried and stored in plastic bags with silica gel  
119 for morpho-anatomical characterization and DNA extraction.

120

## 121 ***2.2 Morpho-anatomical characterization of CCA***

122 For detailed morpho-anatomical characterization, all samples were examined with a scanning  
123 electron microscope (SEM) according to Kaleb et al. (2018). Small fragments of thalli were  
124 mounted on aluminum stubs with acrylic adhesive, coated with a thin layer of chromium (Quorum  
125 Q150T ES coater), and analyzed with a SEM Zeiss Gemini300 at a working distance (WD)  
126 between 8 and 11 mm.

127

## 128 ***2.3 CCA DNA extraction, amplification and sequencing***

129 The silica-dried CCA samples were pulverized using sterile mortars, and the DNA was extracted  
130 following the modified protocol of the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Crawley,  
131 UK) by Broom et al. (2008) and quantified with the use of Qubit dsDNA BR Assay Kit (Invitrogen,  
132 Life Technologies). The psbA gene was PCR-amplified with the use of the forward and reverse  
133 primers psbA-F 5'-ATGACTGCTACTTTAGAAAGACG-3' and psbA-R2 5'-  
134 TCATGCATWACTTCCATACCTA-3' according to Pezolesi et al. (2017, 2019), and the  
135 QIAGEN Multiplex PCR Kit (Qiagen) according to the instructions. The amplification protocol  
136 consisted of a HotStarTaq DNA polymerase activation step at 95°C for 15 min, followed by a 3-  
137 step cycling with denaturation at 94°C for 30 sec, annealing at 57°C for 90 sec, extension at 72°C  
138 for 90 sec for 35 cycles, and a final extension at 72°C for 10 min. The mitochondrial COI-5P  
139 fragment was PCR-amplified in *M. dendrospermum*, to increase the number of reference  
140 sequences available for the subsequent alignment, following Peña et al. (2015), with the use of the

141 forward and reverse primers GazF1 5'-TCAACAAATCATAAAGATATTGG-3' and GazR1 5'-  
142 ACTTCTGGATGTCCAAAAAYCA- 3' (Saunders, 2005). The amplification protocol consisted  
143 of an initial denaturation at 95°C for 2 min followed by 40 cycles of 30 s denaturation at 94°C, 40  
144 s annealing at 50°C and 40 s of extension at 72°C followed by an additional 5 min at 72°C. PCR  
145 products were visualized in 1.5% agarose gels stained with Midori Green using Low DNA Mass  
146 Ladder (Invitrogen, Carlsbad, CA, USA) as a reference, and PCR products with expected lengths  
147 and yields were purified and sequenced by BMR Genomics (Padua, Italy). Specifically, PCR  
148 products were treated with ExoSap (Applied Biosystems™, ThermoFischer Scientific, Walthman,  
149 MA, USA), and sequencing was performed with BrilliantDye Terminator v1.1 Cycle Sequencing  
150 Kit (Nimagen, The Netherlands) following the manufacturer's instruction on a AB3730xl DNA  
151 Analyzer (Applied Biosystems™, ThermoFisher Scientific, Walthman, MA, USA).

152

#### 153 ***2.4 CCA sequence alignments and phylogenetic analyses***

154 The quality of the sequences was assessed by visual inspection of the electropherograms using the  
155 Chromas software (Version 2.6.6; Technelysium Pty LTD, South Brisbane, Australia). All  
156 sequences were scored as high quality, without double or confounding peaks, and were all retained  
157 for the alignment and phylogenetic analyses. Alignment was performed using ClustalW and  
158 default settings in AliView software (Version 1.28; Larsson, 2014) and phylogeny constructed by  
159 means of MEGA software (Version 11.0.13, [www.megasoftware.net](http://www.megasoftware.net); Tamura et al., 2021). In  
160 addition, NCBI database ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) was searched for publicly available sequences  
161 of *Lithophyllum stictiforme* complex and *Mesophyllum* genus (as *Mesophyllum macroblastum* was  
162 only recently recognized as *Macroblastum dendrospermum*, Athanasiadis & Ballantine, 2024),  
163 and some representatives were selected to be included in the alignments. The alignment was

164 assembled after a series of preliminary phylogenetic analyses aimed at correct selection of ingroup  
165 and outgroup taxa. Therefore, *psbA* sequences of *Lithophyllum dentatum* (Kützing) Foslie, *L.*  
166 *hibernicum* Foslie, and *L. bathyporum* Athanasiadis & D.L.Ballantine, and *psbA* and COI-5P  
167 sequences of the Melobesioideae *Phymatolithon calcareum* (Pallas) W.H. Adey & McKibbin and  
168 *Lithothamnion corallioides* (P.L. Crouan & H.M. Crouan) P.L. Crouan & H.M. Crouan were  
169 included as outgroups in the respective data sets, according to previous studies (Pezzolesi et al,  
170 2019; Peña et al., 2015). The sequences' alignment of *L. stictiforme* *psbA* sequences was 825 bp  
171 long, while those of *M. dendrospermum* ranged from 598 (S17R1), 684 (S17R2), 725 (S12R1) to  
172 851 base pairs (bp). The COI-5P of *M. dendrospermum* alignment resulted in 608 bp. Neighbor-  
173 Joining (NJ) distance analyses were performed on all data sets using Maximum Composite  
174 Likelihood model in MEGA software, with nodal support assessed by 1,000 bootstrap (BR)  
175 resamplings. Phylogenetic relationships were inferred using Maximum Likelihood (ML) analyses  
176 in MEGA software, under a generalized time-reversible with gamma+invariant sites heterogeneity  
177 model (GTR+G+I), and a generalized time-reversible gamma distributed (GTR+G) alignments,  
178 for the COI-5P and *psbA* alignments, respectively. The bootstrap consisted of 1000 replicates with  
179 complete deletion option, i.e. eliminating the positions containing gaps and missing data (Saitou  
180 & Nei, 1987; Nei & Kumar, 2000; Pezzolesi et al., 2019).

181 Genetic distances in *Lithophyllum stictiforme* and *Macroblastum dendrospermum* were computed  
182 within and between clades using the Kimura distance (Kimura-2 parameter, Kimura, 1980) from  
183 the alignments in MEGA (v.11.0.13).

184

## 185 ***2.5 Bacteria DNA extraction and sequencing***

186 Frozen CCA samples were pulverized using sterile mortars. Total genomic DNA was extracted  
187 from 18 CCA samples using Power Soil DNA Isolation kit according to the manufacturer's  
188 instructions (QIAGEN, Hilden, Germany). After DNA quantification with the use of DeNovix  
189 DS11 Spectrophotometer - Fluorometer (DeNovix Inc., Delaware), analyses of the prokaryotic  
190 16S rDNA sequences were conducted through amplicons generation with the use of the forward  
191 and reverse primers 341F 5'-CCTACGGGNGGCWGCAG-3' and 785R 5'-  
192 GACTACHVGGGTATCTAATCC-3' amplifying the bacterial V3-V4 region (Klindworth et al.,  
193 2013). Sequencing was performed on the Illumina MiSeq V3 platform at the Integrated  
194 Microbiome Resource (IMR, imr.bio, Dalhousie University, Halifax, Nova Scotia, Canada) to  
195 obtain 2x300 paired-end sequences. Complete details of the protocols applied for library  
196 preparation and sequencing procedures can be found in Comeau and Kwawukume (2023).

197

## 198 ***2.6 Bacteria sequence analyses and statistics***

199 Bacteria sequences were processed with the DADA2 R package, version 1.16 (Callahan et al.,  
200 2016; <https://benjjneb.github.io/dada2/>) in R 4.2.3 (R Core Team, 2023). After inspection of  
201 quality control profiles, the filtering parameters were set as follow: truncLen=c(280,220),  
202 trimLeft=c(17,21), maxN=0, maxEE=c(2,5), truncQ=2, rm.phix=TRUE, compress=TRUE,  
203 multithread=TRUE. The sequences were then dereplicated, denoised by removing sample  
204 inference and chimeras, and merged. Representative amplicon sequence variants (ASVs) were  
205 classified against the SILVA version 138.1 database (McLaren & Callahan, 2021). The dataset  
206 was cleaned from chloroplast, Archaea, unknown ASVs at the phylum level and singletons with  
207 the R package phyloseq (McMurdie & Holmes, 2013). After cleaning, a total of 503,450 reads and  
208 2599 ASVs were retained.

209 Rarefaction curves were produced by rarefying the dataset to 9310 sequences through the  
210 use of *vegan* package in R (Oksanen et al., 2013), and all samples were retained for analyses. The  
211 curves reached a plateau for all samples indicating that the CCA-associated bacteria diversity was  
212 well covered (Supp. Fig. 1).

213 To estimate CCA-bacteria communities alpha diversity, the *phyloseq* package was used to  
214 calculate the Shannon Index with the *diversity* function. Significant differences among alpha  
215 diversity values at both inter and intra-CCA species levels were tested. The Shapiro Wilk test was  
216 applied to test for normality of data. When data were normal, the One-Way ANOVA with *aov*  
217 function followed by the Tukey test for pairwise comparison of samples were used. In case of non-  
218 normal data, the Kruskal-Wallis test followed by the Dunn test (*dunn.test* package) with the  
219 Benjamini-Hochberg method were performed. A multidimensional scaling ordination (MDS),  
220 based on Bray-Curtis distance matrix computed from Hellinger ASV transformed data (i.e., square  
221 root of relative abundance), was constructed with the *vegan* package to visualize similarities in  
222 bacteria community composition both between CCA species and within samples of the same CCA.  
223 A permutational multivariate analysis of variance (PERMANOVA) was used to test for significant  
224 differences among bacteria communities associated with thalli of different and same CCA species  
225 on Bray Curtis distance matrix of Hellinger transformed ASV data using 999 permutations under  
226 reduced model with the *vegan* package by applying the *adonis2* function. Intraspecific differences  
227 of CCA bacteria communities were further analysed by the *pairwise.adonis2* function for pairwise  
228 comparison of samples considering the sampling sites as source of variance. Statistically  
229 significant differences were considered with  $p$ -value  $\leq 0.05$ .

230 To identify the core microbiomes, i.e. ASVs shared between individual CCA-bacteria  
231 communities within and between CCA species, as well as the core ASVs unique to each CCA

232 species and sampling site, i.e. shared only at CCA species level, the microbiome package (Lahti  
233 et al., 2017) and the *core\_members* function (Salonen et al., 2012) with detection = 0.01 (with  
234 relative abundance  $\geq 1\%$ ) and prevalence = 0.75 (present in at least 75% of samples) were used.  
235 The results were represented through Venn diagrams produced with the use of the ggvenn package.  
236 To gain more details on the potential functional traits of the most contributing bacteria taxa among  
237 both the shared and unique ASVs associated to the two CCAs and characteristic of each species,  
238 the five most abundant ASVs were selected for further taxonomic analysis through a manual  
239 BLAST search against the NCBI nucleotide library (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

240 A differential abundance analysis was carried out to compare ASV data from the two CCA  
241 species. The analysis was done by applying DESeq2 analyses (Love et al., 2014) using phyloseq  
242 package in R v.4.4.1 (R Core Team, 2024) with *phyloseq\_to\_deseq2* function followed by *DESeq*.  
243 Significance was determined using a Wald test with a Benjamini and Hochberg adjusted *p*-value.  
244 An adjusted *p*-value  $\leq 0.01$  was used as the minimal cut-off, and analysis results were shown by  
245 selecting the ASVs with a significance  $\leq 0.001$ . The 20 ASVs with the most significant different  
246 abundance were further represented with a heatmap showing also the hierarchical clustering of  
247 samples produced with microViz package and *comp\_heatmap* function on clr transformed data  
248 (Barnett et al., 2021). Based on CCA phylogeny results (see section 3.2), the differential abundance  
249 analysis was carried out on ASVs associated with *L. stictiforme* thalli belonging to two different  
250 clades to deepen the comparison at intraspecific level. Species clade was adopted as comparison  
251 factor and ASVs meeting the minimal cut-off of an adjusted *p*-value  $\leq 0.01$  were considered.

252

### 253 3. Results

254

### 255        **3.1 CCA morpho-anatomy**

256        Based on the morpho-anatomical analyses, all *Lithophyllum stictiforme* individuals were  
257        confirmed to belong to *L. stictiforme* complex (Suppl. Fig. 2A-G) with no substantial differences  
258        in diagnostic features observed across the three sampling sites. The thalli were encrusting with a  
259        free laminar margin and the surface was either flat or slightly undulating. In all individuals, the  
260        epithallus was abraded (Suppl. Fig. 2H), occasionally revealing the underlying cell layer.

261               The *M. dendrospermum* individuals displayed encrusting to warty thalli (Suppl. Fig. 3A)  
262        and exhibited the key diagnostic traits for this species (Suppl. Fig. 3A-H). In surface view, large  
263        areas of shedding epithallial cells were observed in all samples (Fig. 2B), and the epithallial cells  
264        were flared.

265

### 266        **3.2 CCA phylogeny**

267        The molecular phylogenetic analysis based on the *psbA* marker confirmed that all 9 individuals of  
268        *Lithophyllum stictiforme* belonged to the complex, previously described as formed by numerous  
269        clades (Pezzolesi et al., 2019). Clade 3B comprised 6 samples from this study, belonging to two  
270        of the three collection sites, together with some reference sequences from Italy and two from  
271        France, including also one (isolate LLG3464) from the sampling area of the present study (S27  
272        and S7; Fig. 1). Replicates collected at site S25 all belonged to a distinct haplogroup defined as  
273        C3 (de Jode et al., 2019). It contained closely similar reference sequences (99% similarity) from  
274        samples previously collected from the same study area (Banyuls-sur-Mer). The Kimura distances  
275        within clades C3 and 3B were 0.0000, while the highest distance within clades was found for  
276        clades 2 and 5 having a distance of 0.0042 and 0.0051, respectively. The genetic distance between

277 clades C3 and 3B was 0.0180. The mean distance to the reference group (outgroup) was 0.0591  
278 for clade C3 and 0.0550 for clade 3B (Table S1).

279 As for *Macroblastum dendrospermum* thalli, 8 out of the 9 psbA sequences generated in  
280 this study clustered with a Mediterranean *Mesophyllum macroblastum* specimen (voucher  
281 VPF00506, now *Macroblastum dendrospermum*; Athanasiadis & Ballantine, 2024), collected in  
282 the Alboran Sea (Spain), and with the Uncultured Corallinales clone LBC0055, collected at less  
283 than 10 km distance from the area of collection (42.497219 N, 3.135617 E; Bittner et al., 2010;  
284 Fig. 2). The last sequence (S12R2) was closely related to the main cluster. The Kimura distance  
285 within this main group was 0.0018, while the mean distance with S12R2 or the outgroup was  
286 0.0192 and 0.1095, respectively. The mean distance between the 8 specimens and the Uncultured  
287 Corallinales from France (GQ917716) was 0.0009, while with *M. macroblastum* from Spain  
288 (KJ638230) was 0.0028. For the COI-5P marker, only 5 sequences were generated as all  
289 amplification attempts for station 13 samples were unsuccessful. The phylogenetic tree obtained  
290 from the ML analysis of the COI-5P alignment was congruent with the psbA tree (Supp. Fig. 4)  
291 and grouped our sequences within a big clade of Mediterranean *M. macroblastum* with high  
292 support (99%), well separated from the other *Mesophyllum* species (*M. alternans*, *M. expansum*,  
293 *M. lichenoides*, *M. erubescens*, and *M. sphaericum*) (Supp. Fig. 4). The genetic distance within  
294 this group was 0.0125, while with the reference group (outgroup) was 0.2044.

295

### 296 **3.3 Bacterial community diversity associated to CCAs**

297 The average Shannon values ranged between  $4.7 \pm 0.1$  and  $5.5 \pm 0.2$ , and between  $5.7 \pm 0.3$   
298 and  $6 \pm 0.1$  in *L. stictiforme* and *M. dendrospermum* samples, respectively (Fig. 3a). The lowest  
299 values were recorded for *L. stictiforme* taken at site ST27. In *M. dendrospermum* sampling sites

300 ST13 and ST17 values were significantly higher than in ST27 (one of *L. stictiforme* sampling site,  
301  $p$ -value < 0.05\*, Fig. 3a, Table S2). In ST13 the value was also significantly higher than in *L.*  
302 *stictiforme* sampling site ST25 ( $p$ -value < 0.05\*, Fig. 3a, Table S2). Bacteria diversity values at  
303 intraspecific level differed only between individuals belonging to *L. stictiforme* complex,  
304 specifically between ST7 and ST25, and ST7 and ST27 ( $p$ -value < 0.05\* and  $p$ -value < 0.01\*\*,  
305 respectively; Fig. 3a, Table S3). The observed ASV numbers varied between 302 and 898 in the  
306 low diversity site ST25 (*L. stictiforme*; on average 335-600 ASVs among sampling sites), and one  
307 sample at site ST13 (*M. dendrospermum*; on average 626-740 ASVs among sampling sites),  
308 respectively (Fig. 3b).

309 In the MDS ordination of microbial community composition, *L. stictiforme* samples  
310 clustered separately from *M. dendrospermum* ones (Fig. 4). Among *L. stictiforme* samples, those  
311 from ST27 and ST7 were closer to each other than those from ST25. *M. dendrospermum* samples  
312 from sites ST12 and ST17 clustered together more closely than those collected in site ST13.

313 The comparison of CCA-associated bacteria communities, both between samples of the  
314 two CCA species and between samples of the same CCA species collected in different sites,  
315 showed significant differences ( $p$ -value = 0.001\*\*\*, between CCA species;  $p$ -value = 0.005\*\*,  
316 between *L. stictiforme* sampling sites;  $p$ -value = 0.002\*\*, between *M. dendrospermum* sampling  
317 sites; Table S4). Intraspecific pairwise comparison did not capture significant differences between  
318 any sample pair, but higher F values could indicate a larger difference between *L. stictiforme*  
319 bacteria communities in ST7 and ST25, and *M. dendrospermum* bacteria communities in ST12  
320 and ST13, and ST17 and ST13 (Table S6).

321

### 322 ***3.4 CCA bacterial community composition***

323 The taxonomy of CCA-associated bacteria was first analyzed at the class and order levels  
324 considering the five most contributing taxa as relative abundance across the dataset. At the class  
325 level, the bacterial communities of both CCA species were dominated by Alphaproteobacteria,  
326 which accounted for 40% and 46% of the sequences in *L. stictiforme* and *M. dendrospermum*,  
327 respectively (Supp. Fig. 5A, C). They were followed by Gammaproteobacteria (18% and 10%),  
328 Acidimicrobiia (12% and 5%), Planctomycetacia (9% and 18%), and Bacteroidia (5% and 7%).  
329 At the order level, the most abundant group in *L. stictiforme* was Rhizobiales (15%), while in *M.*  
330 *dendrospermum* it was Pirellulales (18%) (Supp. Fig. 5B, D). These two orders were shared among  
331 the two CCAs. Order Rhodobacterales was also shared, while Cellvibrionales and Microtrichales  
332 (8% and 12%), and Flavobacteriales and Rhodovibrionales (6% and 7%) were abundant only in *L.*  
333 *stictiforme* and *M. dendrospermum* bacteria communities, respectively.

334 At the family level, among the 20 most contributing families, Pirellulaceae (phylum  
335 Planctomycetes) were abundant in all CCA bacteria assemblages, contributing on average 4.5-  
336 15% to *L. stictiforme* bacteria communities in ST25 and ST7, and 13-23.4% to *M. dendrospermum*  
337 bacteria communities in ST13 and ST12, respectively (Fig. 5a, b). Shared bacteria families were  
338 also Microtrichaceae (phylum Actinobacteria) and Rhodobacteraceae (class Alphaproteobacteria),  
339 more abundant in *L. stictiforme* (average percentage contribution 7.4-15% in ST7 and ST25  
340 samples, and 5.8-11.4% in ST25 and ST7 samples, respectively), and Kiloniellaceae (class  
341 Alphaproteobacteria) and Flavobacteriaceae (class Bacteroida), more abundant in *M.*  
342 *dendrospermum*, with average contribution of 3.1-12.5% in ST12 and ST13 and 4.1-6.9% in ST13,  
343 respectively.

344 Despite these similarities, differences were observed at the family level both between- and  
345 within-CCA species. Spongiibacteraceae (class Gammaproteobacteria) and Stappiaceaea (class

346 Alphaproteobacteria) were mainly found in *L. stictiforme*. Within this species Spongiibacteraceae  
347 were abundant in ST27 and Stappiaceae in ST25, representing on average 23.2% and 6.8% of the  
348 bacterial community, respectively (Fig. 5a).

349 At the genus level, among the 10 most contributing genera only three were shared between  
350 the two CCA species: undefined Geminococcaceae, *Pir4\_lineage* and *Blastopirellula* (Supp. Fig.  
351 6). All the other genera were uniquely associated with one of the two CCAs. In particular,  
352 undefined Microtrichaceae (phylum Actinobacteria) and BD1-7\_clade were abundant in *L.*  
353 *stictiforme*, while an unknown genus belonging to Alphaproteobacteriaceae and *Bythopirellula*  
354 highly contributed to *M. dendrospermum* microbiome (Supp. Fig. 6).

355

### 356 **3.5 Core shared and unique and differently abundant bacteria**

357 A total of 65 ASVs (2.5% of the ASVs) constituted the core microbiome (ASVs present in at least  
358 75% of samples) shared between *L. stictiforme* and *M. dendrospermum* (Fig. 6a). A total of 86  
359 (3.3%) and 154 (5.9%) ASVs were uniquely associated to the core microbiome of *L. stictiforme*  
360 and *M. dendrospermum*, respectively. When comparing sampling sites, in *L. stictiforme*, 23 ASVs  
361 (0.5% of the total ASVs of *L. stictiforme* bacteria) were present as core taxa at all sites, with sites  
362 ST7 and ST27 further sharing the highest number of ASVs (20) (Fig. 7b). For individuals collected  
363 in the same site, 4.2 to 7.5% of core ASVs were shared among samples of ST27 and among  
364 samples of ST25, respectively (Fig. 6b). In *M. dendrospermum*, 84 core ASVs (1.4% of the total  
365 *M. dendrospermum* bacteria ASVs) were found in all sites, and ST12 and ST17 further shared the  
366 most core ASVs (40). For *M. dendrospermum* samples collected in the same site, the percentage  
367 of core ASVs ranged between 11.3-15.4% among samples of ST12 and ST13, respectively (Fig.  
368 6c).

369           Among the core ASVs, ASVs 13 and 24 were identified as belonging to *Filomicrobium*  
370 species, ASV 39 was classified as *Ruegeria atlantica*, ASV 48 was ascribed to  
371 *Sva0996\_marine\_group*, and ASV 14 belonged to the family Rhizobiaceae (Table 1). Among the  
372 core ASVs unique to each CCA species, ASVs of the genera *Pir4\_lineage* and *Blastopirellula*  
373 were present in all CCA bacteria assemblages.

374           A total of 152 ASVs were differently abundant between CCA species. Among them, 89  
375 were the most significantly different, meeting an adjusted  $p$ -value  $\leq 0.001^{***}$ , and belonged  
376 mainly to the families Pirellulaceae, Rhodobacteraceae, Flavobacteriaceae and Rubritaleaceae  
377 (Fig. 7a). Among these, the 20 most differently abundant showed distinct distributional patterns  
378 between CCA species that clustered separately (Fig. 7b), and fell within the list of non-shared  
379 ASVs between the two algal species (Table 1). The complete list of differently abundant ASVs  
380 between CCA species is reported in Table S7.

381           Between *L. stictiforme* clades 3B and C3, 17 ASVs were significantly different in  
382 abundance, among which ASV876 Desulfobulbaceae, ASV686 Microtrichaceae, ASV47 and  
383 ASV212 Geminococaceae presented the highest log<sub>2</sub> fold change values, and ASV20  
384 Prochloraceae, ASV9 undefined Gammaproteobacteria and ASV1 Spongiibacteraceae presented  
385 the lowest ones (Fig. 8a). Distinct distributional patterns of ASVs between clades are shown in  
386 Fig. 8b. Samples belonging to the two clades clustered separately, as well as samples of clade 3B  
387 when comparing ST7 and ST27 sampling sites. Detailed results are reported in Table S8.

388

#### 389           **4. Discussion**

390

391 The results from both morpho-anatomical and molecular taxonomy confirmed the assignment of  
392 the analyzed CCA thalli to *Lithophyllum stictiforme* and *Macroblastum dendrospermum*. Our  
393 study shows that individuals belonging to diverse haplotypes of the *L. stictiforme* complex can  
394 coexist within a restricted area. Indeed, molecular taxonomy results revealed that two of the three  
395 sampling sites hosted individuals of clade 3B, while the CCAs from ST25 belonged to haplotype  
396 C3. The latter haplotype was previously and uniquely identified in the same sampling area  
397 (Pezzolesi et al., 2019; de Jode et al., 2019), indicating a possible Banyuls endemism.

398 The bacterial assemblages associated with the CCA samples showed similar or higher  
399 diversity compared to CCA microbiomes from other marine regions (e.g., Barott et al., 2011;  
400 Hester et al., 2016 and Yang et al., 2021). Mediterranean CCAs may therefore represent a reservoir  
401 of bacterial diversity, emphasizing the need for further research on the role of microbial  
402 communities in coralligenous habitat functioning.

403 In both CCA species, Proteobacteria dominated the bacterial communities, especially the  
404 Alpha and Gammaproteobacteria classes, followed by Planctomycetacia, Acidimicrobiia and  
405 Bacteroidia. These groups have been frequently detected in most of the investigated CCAs (Barott  
406 et al., 2011; Sneed et al, 2015; Brodie et al., 2016; Cavalcanti et al., 2018; Gefen-Treves et al.,  
407 2021). At the order level, Rhodobacterales, Rhizobiales and Rhodovibrionales (all  
408 Alphaproteobacteria) were among the dominant taxa, which is consistent with previous studies  
409 (Cavalcanti et al., 2014; Hester et al., 2016; Quéré et al., 2019). This shows that Mediterranean  
410 CCAs host bacterial communities that are taxonomically similar to those of other CCAs  
411 worldwide, at least at the class and order levels. In our study, the dominant bacterial families were  
412 also the most shared among the CCAs. Microtrichaceae and Rhodobacteriaceae, which were  
413 abundant in *L. stictiforme* individuals, were also well represented in *M. dendrospermum*, while

414 Kiloniellaceae and Flavobacteriaceae, which predominate in *M. dendrospermum*, were also  
415 abundant in *L. stictiforme* bacterial communities. Nonetheless, bacterial communities were found  
416 to differ between the two CCA species, with *M. dendrospermum* exhibiting higher bacterial alpha  
417 diversity based on the Shannon index. Studies from other regions have similarly reported species-  
418 specific microbiomes in other CCA species (e.g., see Barrott et al., 2011 - Caribbean; Sneed et al.,  
419 2015 - Belize; Brodie et al. 2016 - UK). At the family level, the bacterial taxa that contributed  
420 differentially to CCAs included Thiohalorhabdaceae and Illumatobacteraceae, which were  
421 exclusively associated with *L. stictiforme*, and Desulfobulbaceae and NB1-j, which occurred only  
422 in *M. dendrospermum*. These families were also unevenly distributed among host individuals.  
423 Indeed, bacterial communities differed not only between CCA species, but also between  
424 individuals of the same species, even if they were collected within a small geographical area (ca.  
425 30 m<sup>2</sup>). CCA intraspecific variability of bacterial communities has previously been linked to  
426 geographical distance, depth gradient, seasonality, and anthropogenic influences (Gefen-Treves et  
427 al., 2021). Host genotype can also influence microbial diversity, as shown by studies on seaweeds  
428 (Wood et al., 2022; Vadiillo Gonzalez et al., 2023). However, environmental conditions often play  
429 a more important role than genotype in shaping holobiont microbial communities, driving adaptive  
430 responses in the microbiome (Pearman et al., 2024; Wood et al., 2022; Baldassarre et al., 2023;  
431 Dubé et al., 2021).

432 Remarkably, we found two highly predominant bacteria families exclusively present in *L.*  
433 *stictiforme*, namely Spongiibacteriaceae and Stappiaceae. Spongiibacteriaceae occurred almost  
434 exclusively in clade 3B of ST27, while Stappiaceae was predominant only in clade C3.  
435 Spongiibacteriaceae are Gammaproteobacteria potentially able to use proteorhodopsin to exploit  
436 light as an additional energy source (Holert et al., 2018). This group was found in association with

437 the CCA *Neogoniolithon trichotomum* in the Gulf of California, at high temperature and silicate  
438 concentration (Valdespino-Castillo et al., 2021), and in Mediterranean sponges as steroid-  
439 degrading bacteria (Spring et al., 2015). In this sense, steroids may act as immune defense boosters  
440 under thermal stress and they have been associated to anti-inflammatory, antioxidant and  
441 antifouling effects in corals (Kong et al., 2012; Dembitsky, 2023; Ochsenkühn et al., 2023). They  
442 can also be produced by the tropical CCA *Hydrolithon boergesenii*, potentially acting as triggers  
443 for coral larval settlement (Quinlan et al., 2023). Stappiaceae have been found associated with  
444 benthic organisms (e.g., sponges, corals, anemones). They have genetic traits that hint to a possible  
445 role in sulfur cycling and dimethylsulfoniopropionate (DMSP) production, a scavenge ROS  
446 compound with antioxidant potential, and in increased abundance in response to thermal stress  
447 (Couceiro et al., 2021; Heric et al., 2023). The reason behind the selective abundance of these two  
448 families in two of the three *L. stictiforme* sampling sites remains unclear. We can hypothesize that  
449 the effects of the individual genotype on the microbial communities' composition, combined with  
450 microenvironmental variations (e.g., light and nutrients availability, presence of localized  
451 stressors) within and around CCA tissues, (see for instance Valdespino-Castillo et al., 2021), cause  
452 the observed differences between individuals of the same species. Furthermore, different thalli  
453 may vary in age, possibly influencing the diversity of the microbiome that has been shown to  
454 increase with age (Bengtsson et al., 2012; Sneed et al., 2015). Differential predation pressure on  
455 CCA thalli may also induce the proliferation of protective, biofilm-forming bacteria (Matz et al.,  
456 2008).

457         At the ASV level, only 2.5% of the total ASVs were shared between CCA species, while  
458 those that were differentially abundant corresponded to the 5.9%. The number of shared ASVs  
459 between individuals of the same CCA species was also low compared to previous studies (Miranda

460 et al., 2013; Brodie et al., 2016; Valdespino-Castillo et al., 2021). In *L. stictiforme*, not only few  
461 ASVs were shared between taxonomic clades, but also between thalli of the same clade.  
462 Hierarchical clustering on differently abundant ASVs showed *L. stictiforme* samples clustering  
463 primarily in relation to the clade they belonged to, and subsequently according to the sampling  
464 site. Indeed, these ASVs differently enriched the bacterial communities associated with clade 3B  
465 thalli collected in different sampling sites. These results further indicated that both genetic and  
466 environmental factors drive the composition of bacterial associations with CCAs, not only at  
467 species but also at haplotype level, and that CCA holobionts can possess a high level of adaptation  
468 to environmental changes. The percentage of shared ASVs only increased (4.2-7.5%) when  
469 individuals collected from the same sampling sites were compared. These results suggest a third  
470 potential driver in holobiont composition: the physical proximity between hosts may promote the  
471 development of more similar bacterial assemblages. We can hypothesize that this proximity effect  
472 could be due to bacteria horizontal transfer (Hester et al., 2016). These hypotheses need to be  
473 further investigated by specifically targeting these bacteria.

474         Among the core bacteria, we found ASVs shared among individuals of the two species.  
475 These ASVs were similar to some earlier found in biofilms, but also in endolithic communities of  
476 marine outcrops or in sponges and seaweeds (see Table 1). Diverse ASVs of *Blastopirellula* and  
477 *Pir4\_lineage* fell within the list of top five abundant ASVs of both CCA species. Planctomycetes  
478 are commonly associated with red algae being able to resist the action of several algal antimicrobial  
479 compounds, and to live in biofilms taking advantage of the polysaccharides commonly produced  
480 by the algae, especially sulphated heteropolysaccharides (agars and carrageenans) (Lage &  
481 Bondoso, 2014; Wegner et al. 2013; Bergstrom et al., 2023). In particular, *Blastopirellula* and the  
482 *Pir4\_lineage* can exploit such compounds thanks to sulfatase enzymes (Bondoso et al., 2017).

483 Interestingly, sulfated glucosaminoglycan, together with other glucosamine and galactose  
484 residues, can be produced by certain CCAs and act as potential triggers for coral larvae settlement  
485 and metamorphosis (Morse & Morse, 1991). Although these ASVs were not shared between the  
486 two CCA species, they were predominantly shared among individuals either of *L. stictiforme* and  
487 *M. dendrospermum*. These findings suggest that the facilitative interaction between Mediterranean  
488 CCAs and gorgonians could be mediated by bacteria, deserving further investigation.

489

## 490 **5. Conclusions**

491 Our results showed that both *Litophyllum stictiforme* and *Macroblastum dendrospermum* host  
492 holobiont-specific bacterial communities, potentially serving as reservoirs of bacterial diversity.  
493 The composition of these microbial communities differed also among individual CCA thalli. We  
494 hypothesize three main drivers of such differences: i) microbiome associations linked to host  
495 genotype, ii) adaptation of bacterial communities to exogenous factors acting at the scale of  
496 individual thalli, and iii) potential host-to-host bacteria transfer between holobionts in close  
497 proximity. These hypotheses should be further tested by examining bacterial communities  
498 responses to stress conditions, both in field and through manipulative experiments, also exploring  
499 their possible seasonal and geographical variability. In the two CCA species studied, we identified  
500 predominant bacterial genera affiliated with taxa known to potentially promote coral larval  
501 settlement in tropical environments. These findings offer promising insights for future research on  
502 the positive interaction between CCA holobionts and coral larvae, mediated by bacteria, in the  
503 Mediterranean Sea.

504

## 505 **Declaration of interests**

506 The authors declare no competing financial or non-financial interests.

507

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515

## 516 **Supplementary Information**

517 The link to the electronic supplementary material can be found online. Crustose coralline algae  
518 (CCAs) and bacterial sequences are accessible at the European Nucleotide Archive (ENA;  
519 <https://www.ebi.ac.uk/ena/browser/home>) under the project accession numbers PRJEB83080  
520 and PRJEB82879, respectively.

521

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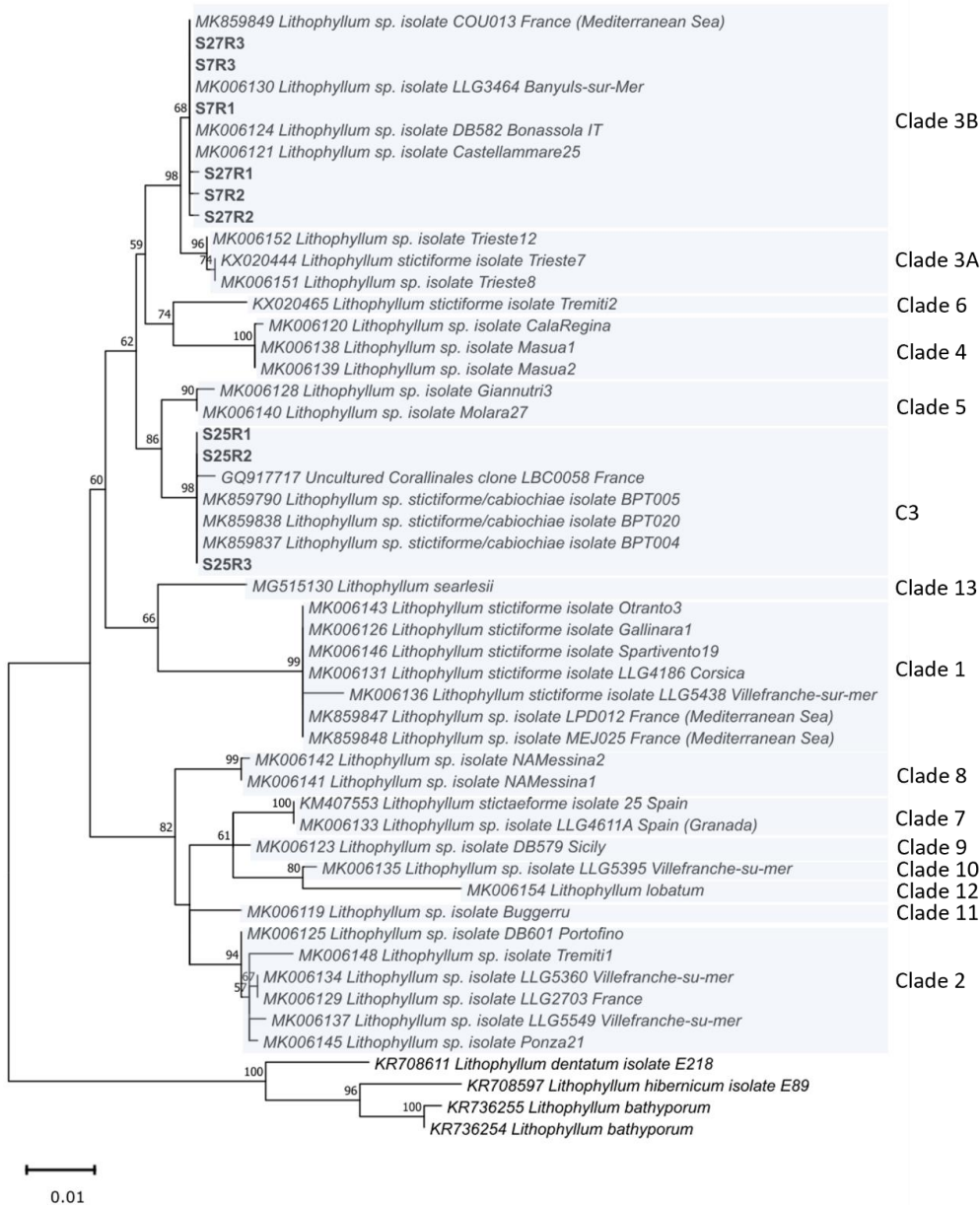
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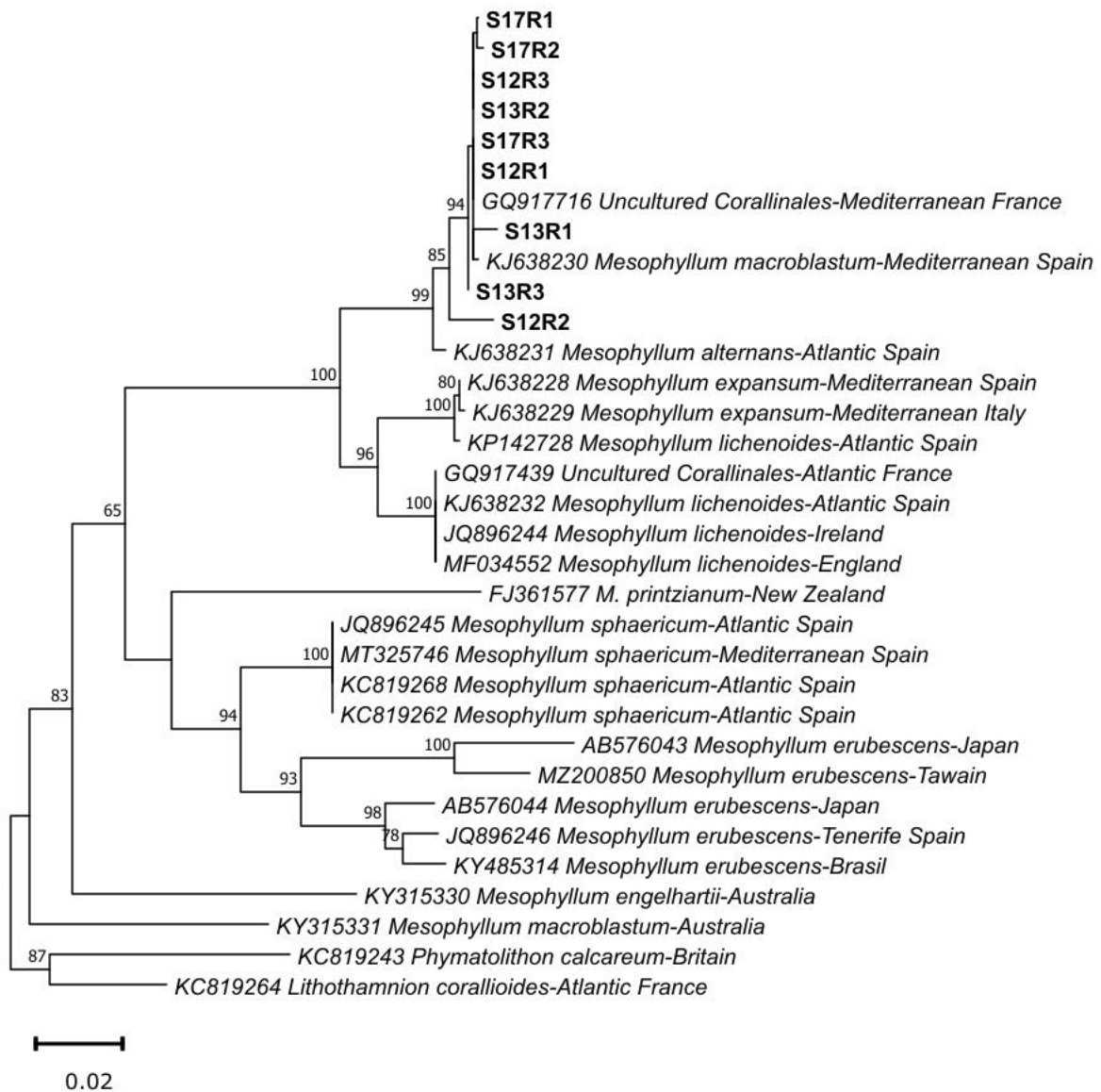
848 **Figures and Table**

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851 **Fig. 1** *Lithophyllum stictiforme* phylogenetic tree. Phylogenetic tree inferred from ML analysis of the psbA  
 852 sequences of Mediterranean *Lithophyllum stictiforme* and other publicly available sequences for this  
 853 complex. Bootstrap ML values > 60% are shown for each node. *L. dentatum*, *L. hibernicum* and *L.*  
 854 *bathyporum* were used as outgroup. Clades 1 to 13 refer to the classification provided by Pezzolesi et al.  
 855 (2019), while C3 corresponds to the haplotype identified by de Jode et al. (2019). Scale bar: 0.01  
 856 substitutions per site. Sequences generated in the present work are marked in bold



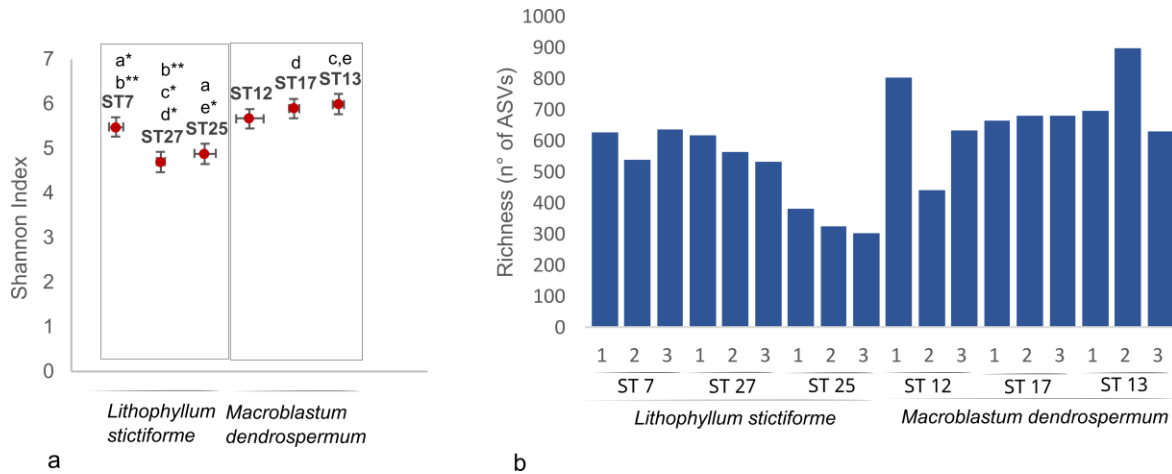
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858 **Fig. 2** *Macroblastum dendrospermum* phylogenetic tree of the psbA sequences. Phylogenetic tree inferred  
 859 from ML analysis of the psbA sequences of Mediterranean *Mesophyllum* and other publicly available  
 860 sequences for this genus as *Mesophyllum macroblastum* was only recently recognized as *Macroblastum*  
 861 *dendrospermum* (Athanasiadis and Ballantine, 2024). Bootstrap ML values > 60% are shown for each node.  
 862 Members of the subfamily Melobesioideae were used as outgroup. Scale bar: 0.02 substitutions per site.  
 863 Sequences generated in the present work are marked in bold

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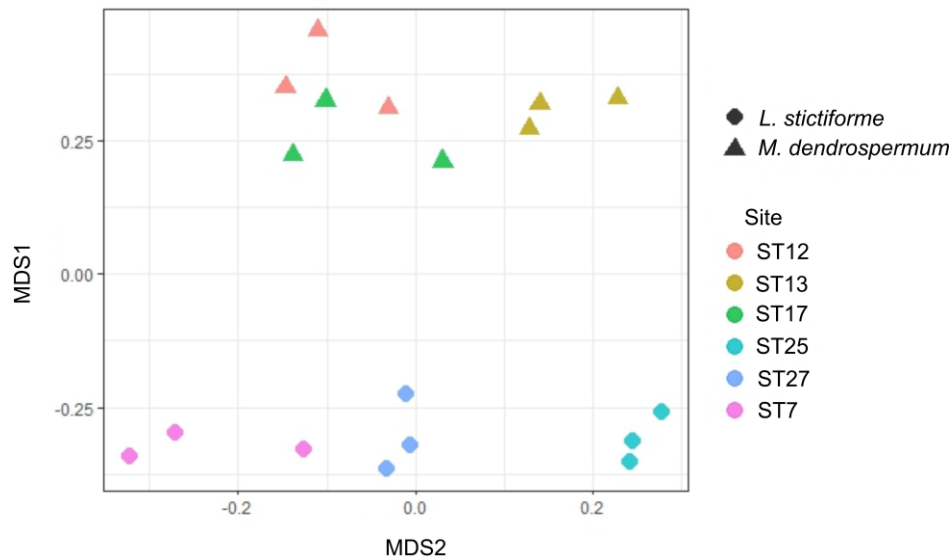
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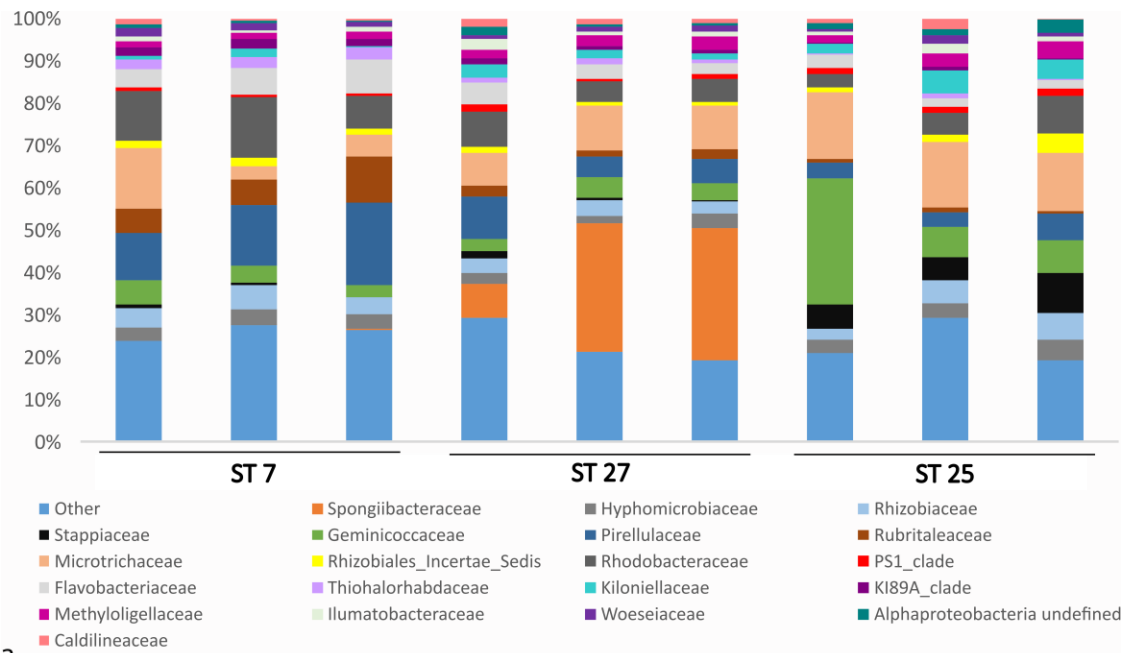
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**Fig. 3** Alpha diversity of bacterial communities associated with the CCA *Lithophyllum stictiforme* and *Macroblastum dendrospermum*. **a.** Mean values of the Shannon index in prokaryotic communities associated with CCAs from the six sampling sites. Significant differences are reported coded by letter and "\*" (p-value < 0.05). **b.** Bacterial community richness (as observed ASVs) reported in each CCA individual.

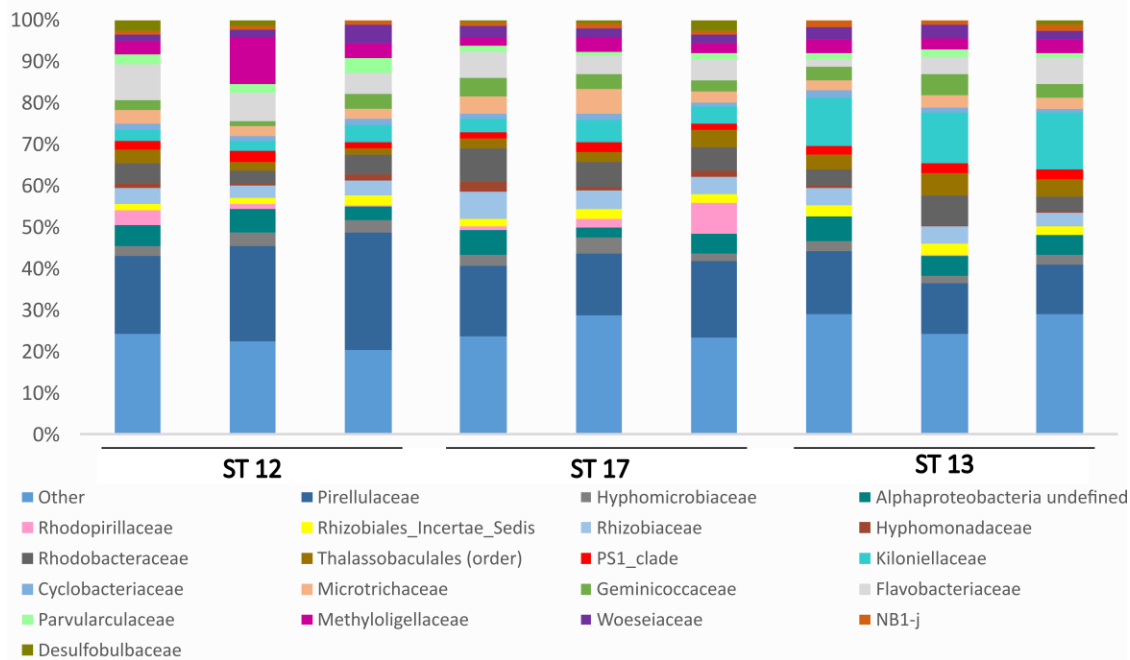


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**Fig. 4** Beta diversity of bacterial communities associated with CCA hosts. Multidimensional scaling ordination (MDS) based on Bray-Curtis distance matrix computed from Hellinger transformed data showing the distribution and dispersion of bacteria communities associated with the CCAs *Lithophyllum stictiforme* and *Macroblastum dendrospermum* sampled at different sites at the ASV level.



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b

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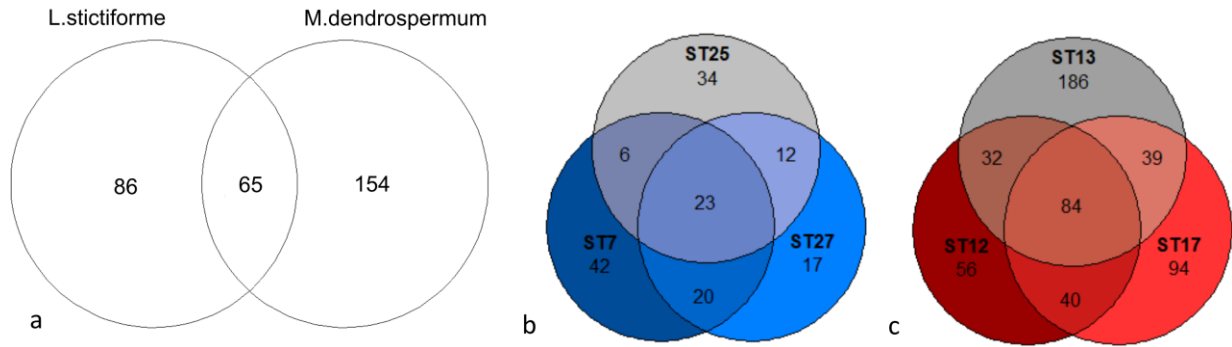
885 **Fig. 5** CCA bacteria taxonomy at the family level. Relative abundance of the 20 most abundant bacteria  
 886 families associated with the two CCA species: **a.** Individuals of *Lithophyllum stictiforme* and **b.** of  
 887 *Macroblastum dendrospermum* presented by site.

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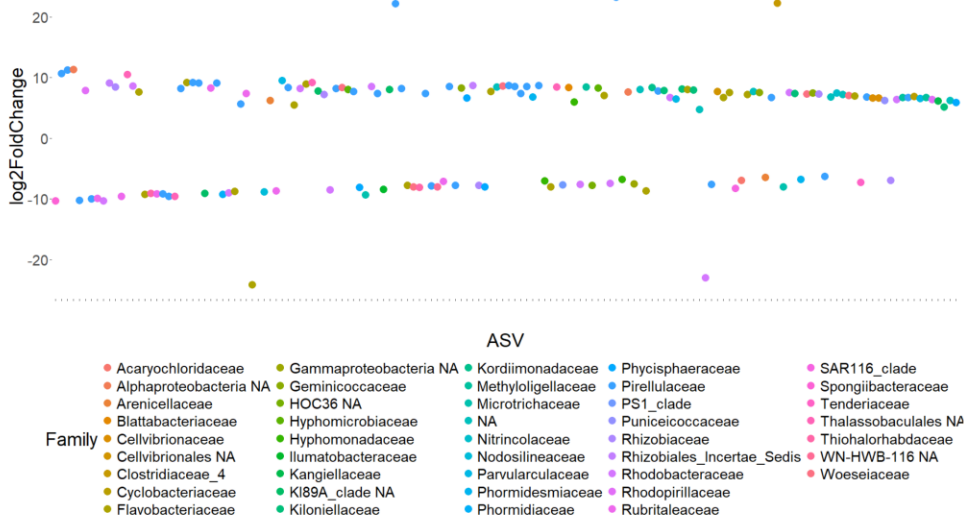


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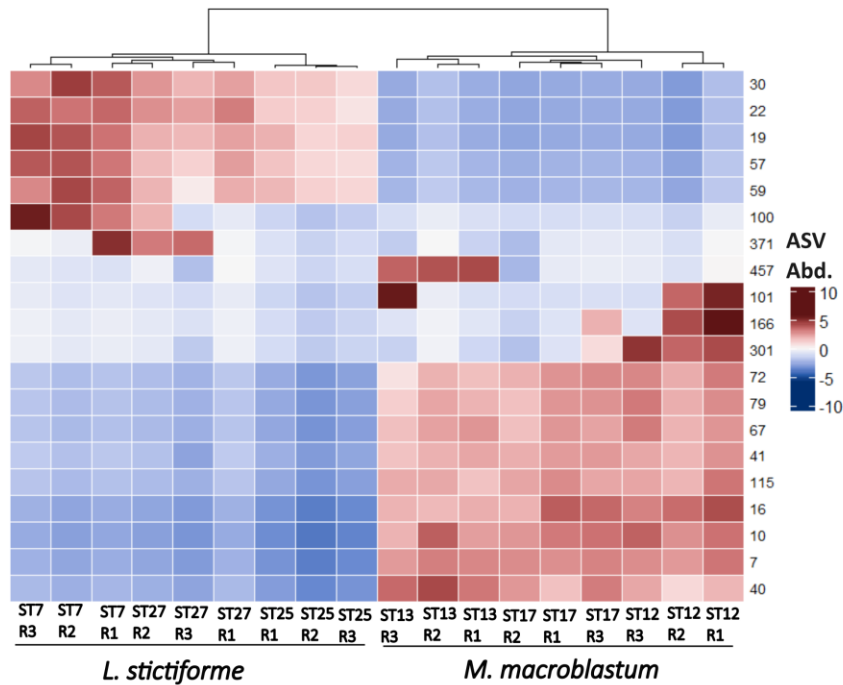
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894 **Fig. 6.** Venn diagrams of shared core microbiomes. **a.** Core ASVs at 75% prevalence shared between  
895 bacteria communities associated with *Lithophyllum stictiforme* and *Macroblastum dendrospermum*  
896 samples. **b.** Core ASVs at 75% prevalence shared between bacteria communities associated with  
897 *Lithophyllum stictiforme* at the three sampling sites. **c.** Core ASVs at 75% prevalence shared between  
898 bacteria communities associated with *Macroblastum dendrospermum* at the three sampling sites.

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a

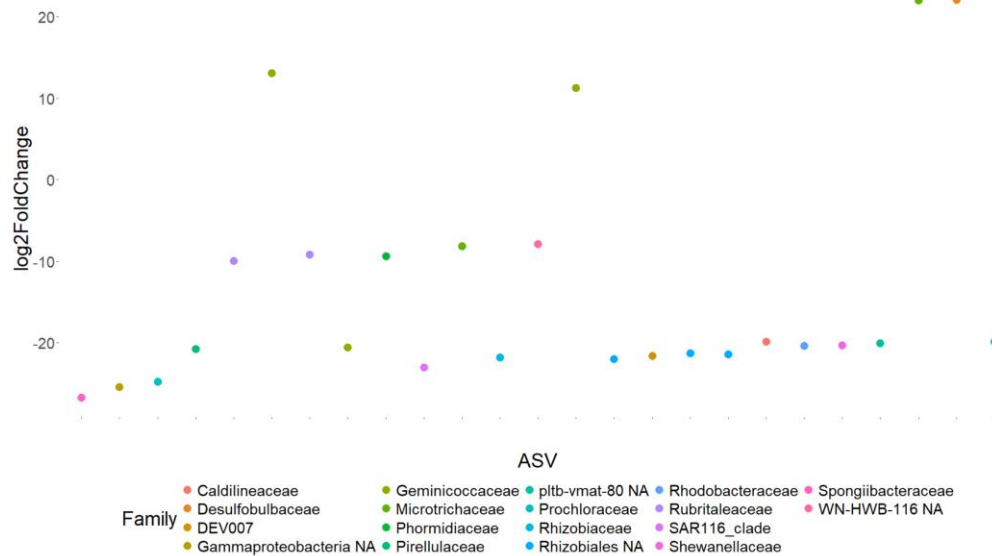


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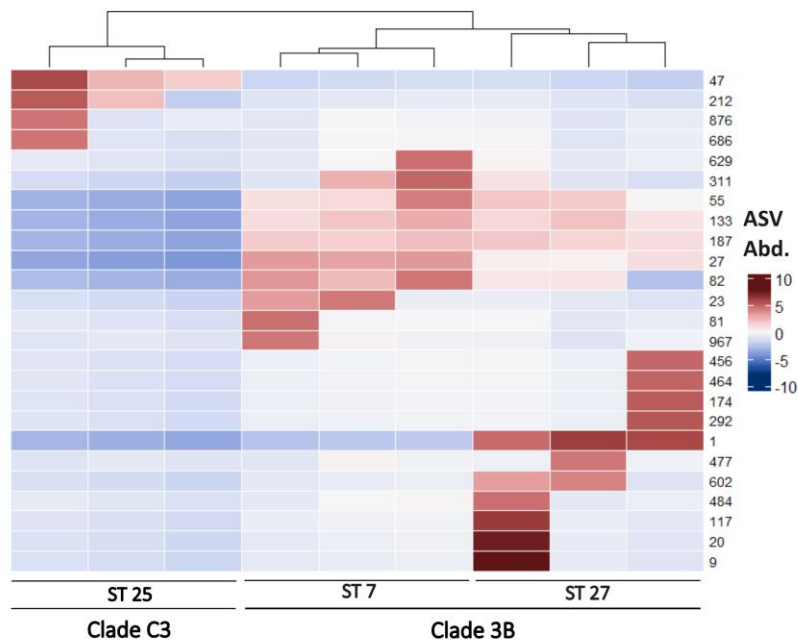
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901 **Fig. 7.** Differently abundant ASVs between CCA species. **a.** Only ASVs presenting adjusted  $p$ -value  
 902  $\leq 0.001$  are shown. The bacterial families to which the ASVs belong are reported in the legend. **b.** The 20  
 903 ASVs most significantly different in abundance, with a significance  $\leq 0.001$ , are represented in the a  
 904 heatmap.

905



a



b

906

907 **Fig. 8.** Differently abundant ASVs between *L. stictiforme* clades. **a.** ASVs presenting adjusted  $p$ -value  
 908  $\leq 0.01$  are shown. The bacterial families to which the ASVs belong are reported in the legend. **b.** ASVs are  
 909 represented in the heatmap.

910

911 **Table 1** Five most abundant shared and unique bacteria ASVs. ASVs are shown per category: shared  
 912 between all CCA, all *Lithophyllum stictiforme* specimens and all *Macroblastum dendrospermum*  
 913 specimens, and unique to all *Lithophyllum stictiforme* and all *Macroblastum dendrospermum* specimens

	ASV	Order	Family	Species	Best match NCBI	Similarity	Origin	Reference
ASVs shared between the two CCA taxa bacteria communities	ASV 13	Rhizobiales	Hyphomicrobiaceae	Filomicrobium sp.	KF185466	100%	marine snow in North Adriatic Sea	Vojvoda et al., 2014
	ASV 14	Rhizobiales	Rhizobiaceae	NA	JQ236006	100%	biofilms from water purification system	Manes et al., 2011
	ASV 24	Rhizobiales	Hyphomicrobiaceae	Filomicrobium sp.	JX984050	100%	biofilms implicated in <i>M. edulis</i> settlement	Toupoint et al., 2012
	ASV 39	Rhodobacterales	Rhodobacteraceae	Ruegeria atlantica	MN704271	100%	sponges from the central coastal region of Vietnam	Dat et al., 2021
	ASV48	Microtrichales	Microtrichaceae	Sva0996_marine_group	JN113050	100%	marine sponge <i>Astroclera willeyana</i>	Yang & Li, 2012
ASVs unique of <i>L. stictiforme</i> bacteria communities	ASV 19	Pirellulales	Pirellulaceae	Pir4_lineage sp.	EU918001	98,32%	biofilm on artificial microbialites and natural stromatolites	Havemann et al., 2008
	ASV 22	Pirellulales	Pirellulaceae	Blastopirellula sp.	KT973101	100%	endolithic communities from intertidal outcrops	Couradeau et al., 2017
	ASV 30	Rhodobacterales	Rhodobacteraceae	Silicimonas sp.	MK175845	99,75%	coral <i>Astrangia poculata</i> microbiome	Goldsmith et al., 2019
	ASV 54	Thiohalorhabdales	Thiohalorhabdaceae	Granulosicoccus sp.	KY468648	96,49%	oil-polluted subtidal sediments North Spain coast	Acosta-González et al., 2013
	ASV 57	Pirellulales	Pirellulaceae	Pir4_lineage sp.	FJ652505	98,8%	sponge <i>Mycale laxissima</i>	Mohamed et al., 2010
ASVs unique of <i>M. dendrospermum</i> bacteria communities	ASV 7	Pirellulales	Pirellulaceae	Pir4_lineage	HM596354	98,56%	biofilm on condenser tube surfaces in a nuclear power plant	Choi et al., 2010
	ASV 34	Rhizobiales	Rhizobiaceae	NA	KT973129	97,76%	endolithic communities from marine intertidal outcrops	Couradeau et al., 2017
	ASV 41	Rhodobacterales	Rhodobacteraceae	Roseovarius sp.	KT973898	99%	endolithic communities from marine outcrops	Couradeau et al., 2017
	ASV 63	Pirellulales	Pirellulaceae	Blastopirellula sp.	EF215770	98,01%	surface biofilm in biofilm formation process in the West Pacific coast	Dang et al., 2008
	ASV 67	Pirellulales	Pirellulaceae	Pir4_lineage	FJ652505	98,08%	marine sponge <i>Mycale laxissima</i>	Mohamed et al., 2010
ASVs shared between <i>L. stictiforme</i> bacteria communities	ASV 13	Rhizobiales	Hyphomicrobiaceae	Filomicrobium sp.	KF185466	100%	marine snow in North Adriatic Sea	Vojvoda et al., 2014
	ASV 15	Rhizobiales	Stappiaceae	Breoghanian sp.	HM177744	100%	CCA <i>Neogoniolithon fosliei</i>	Webster et al., 2011
	ASV 19	Pirellulales	Pirellulaceae	Pir4_lineage sp.	EU918001	98,32%	biofilm on artificial microbialites and natural stromatolites	Havemann et al., 2008
	ASV 22	Pirellulales	Pirellulaceae	Blastopirellula sp.	KT973101	100%	endolithic communities intertidal outcrops	Couradeau et al., 2017
	ASV 30	Rhodobacterales	Rhodobacteraceae	Silicimonas sp.	MK175845	99,75%	coral <i>Astrangia poculata</i> microbiome	Goldsmith et al., 2019
ASVs shared between <i>M. dendrospermum</i> bacteria communities	ASV 6	Pirellulales	Pirellulaceae	Bythopirellula sp.	JN596618	99,29%	tropical sponges, <i>Xestospongia muta</i> and <i>Xestospongia testudinaria</i>	Montalvo et al., 2011
	ASV 7	Pirellulales	Pirellulaceae	Pir4_lineage	HM596354	98,56%	biofilm on condenser tube surfaces in nuclear power plant	Choi et al., 2010
	ASV 13	Rhizobiales	Hyphomicrobiaceae	Filomicrobium sp.	KF185466	100%	marine snow in North Adriatic Sea	Vojvoda et al., 2014
	ASV 14	Rhizobiales	Rhizobiaceae	NA	JQ236006	100%	biofilms from water purification system	Manes et al., 2011
	ASV 23	Pirellulales	Pirellulaceae	Blastopirellula sp.	HM369093	99,26%	biofilms on surfaces of the kelp <i>Laminaria hyperborea</i>	Bengtsson et al., 2010