

# 1      **Genomic adaptations to an endolithic lifestyle in** 2                      **the coral-associated alga *Ostreobium***

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25

## 26 **Abstract**

27 The green alga *Ostreobium* is an important coral holobiont member, playing key roles in  
28 skeletal decalcification and providing photosynthate to bleached corals that have lost their  
29 dinoflagellate endosymbionts. *Ostreobium* lives in the coral's skeleton, a low-light environment  
30 with variable pH and O<sub>2</sub> availability. We present the *Ostreobium* nuclear genome and a  
31 metatranscriptomic analysis of healthy and bleached corals to improve our understanding of  
32 *Ostreobium*'s adaptations to its extreme environment and its roles as a coral holobiont member.  
33 The *Ostreobium* genome has 11,735 predicted protein-coding genes and shows adaptations for  
34 life in low and variable light conditions and other stressors in the endolithic environment. It  
35 presents an unusually rich repertoire of light harvesting complex proteins, lacks many genes for  
36 photoprotection and photoreceptors, and has a large arsenal of genes for oxidative stress  
37 response. An expansion of extracellular peptidases suggests that *Ostreobium* may supplement  
38 its energy needs and compensate for its lysine auxotrophy by feeding on the organic skeletal  
39 matrix, and a diverse set of fermentation pathways allow it to live in the anoxic skeleton at  
40 night. *Ostreobium* depends on other holobiont members for biotin and vitamin B12, and our  
41 metatranscriptomes identify potential bacterial sources. Metatranscriptomes showed *Ostreobium*  
42 becoming a dominant agent of photosynthesis in bleached corals and provided evidence for  
43 variable responses among coral samples and different *Ostreobium* genotypes. Our work  
44 provides a comprehensive understanding of the adaptations of *Ostreobium* to its extreme  
45 environment and an important genomic resource to improve our comprehension of coral  
46 holobiont resilience, bleaching and recovery.

47

## 48 **Introduction**

49 Coral health depends on the harmonious association between the coral animal and its  
50 microbial associates, together known as the holobiont. A wealth of studies shows that climate  
51 change threatens coral health by disrupting the association between the coral and its

52 photosynthetic dinoflagellate endosymbionts (Symbiodiniaceae), culminating in coral bleaching  
53 and death. The role of other microbes in coral resilience is just starting to be understood, and  
54 our current knowledge is largely based on correlations between coral health status and the  
55 presence of microbial taxa inferred from metabarcoding. Whole-genome sequences of corals  
56 and their symbionts are an invaluable resource to obtain a mechanistic understanding of the  
57 functioning and resilience of the holobiont. Recent genomic studies have shown that the coral  
58 host, dinoflagellate symbionts, and prokaryotes living in the coral tissue have complementary  
59 pathways for nutrient exchange, highlighting the interdependence and possible co-evolution  
60 between these members of the coral holobiont<sup>1,2</sup>. To date, most work has focused on the coral  
61 animal and microbiota associated with its living tissue, with very little work done on the highly  
62 biodiverse and functionally important microbiota inhabiting the skeleton of the coral<sup>3,4</sup>.

63 The green alga *Ostreobium* sp. is an important eukaryotic symbiont living inside the coral  
64 skeleton (Fig. 1a)<sup>4,5</sup>. This endolithic alga is the principal agent of coral reef bioerosion,  
65 burrowing through the limestone skeleton of corals and other reef structures and dissolving up  
66 to a kilogram of reef CaCO<sub>3</sub> per m<sup>2</sup> per year<sup>6</sup>. These algae bloom in the skeleton when corals  
67 bleach (Fig. 1b)<sup>7,8</sup>, boosted by the extra light, and – hypothetically – the extra nitrogen and CO<sub>2</sub>  
68 that may reach the skeleton in the absence of Symbiodiniaceae. Part of the carbohydrates  
69 produced by *Ostreobium* photosynthesis make their way into the coral tissue, extending the time  
70 it can survive without its dinoflagellate partner<sup>9,10</sup>. While these ecological and physiological  
71 phenomena have been described, our knowledge of the molecular mechanisms involved is  
72 scarce, severely limiting our understanding of key processes in healthy and bleached holobionts.  
73 What is the mechanism of skeletal deterioration and how tightly does *Ostreobium* metabolism  
74 integrate with that of the coral and associated microbiota? Developing this knowledge will be  
75 essential to understand and manage the roles that skeletal microbiota play during coral  
76 bleaching.

77 *Ostreobium* has an extreme lifestyle for a green alga<sup>11</sup>. It lives in a very dimly lit  
78 environment, with mostly the low-energy far-red wavelengths not used up by Symbiodiniaceae  
79 available<sup>12</sup>. The action spectrum of *Ostreobium* photosynthesis extends into far-red

80 wavelengths but the underlying molecular mechanisms are little known<sup>13,14</sup>. The daily rhythm  
81 of oxygenic photosynthesis and respiration within the skeletal matrix leads to strong  
82 fluctuations of pH and O<sub>2</sub>, ranging from total anoxia at night to ca. 60% of air saturation during  
83 the day<sup>11,15</sup> and the skeleton limits diffusion of O<sub>2</sub> and other compounds. These stressful  
84 conditions are not normally encountered by free-living algae and the mechanisms allowing  
85 *Ostreobium* to thrive in this extreme habitat are virtually unknown.

86 From an evolutionary perspective, *Ostreobium* is a member of the green plant lineage  
87 (Viridiplantae), which includes the land plants that originated in the Streptophyta lineage and a  
88 broad diversity of algae in the Chlorophyta lineage<sup>16</sup> (Fig. 1c). *Ostreobium* is in the  
89 Bryopsidales, an order of marine algae that has evolved in the Chlorophyta. While *Ostreobium*  
90 forms small microscopic filaments, many representatives of this order are larger seaweeds<sup>17</sup>.

91 So far, the genomic resources available for coral holobiont research have been limited to  
92 the coral animal (7 genomes), its dinoflagellate photosymbionts (14 genomes) and a small  
93 fraction of the prokaryotes associated with its tissue (52 metagenome-assembled genomes<sup>2</sup>).  
94 Here, we present the first nuclear genome of an *Ostreobium* species to extend the available  
95 genomic toolkit into the coral skeleton, an element of the holobiont crucial for our  
96 comprehension of coral resilience, bleaching, and recovery. We expect that these genomic  
97 resources will spur new insights into processes of coral bleaching encompassing the entire  
98 holobiont, as this knowledge will be essential to safeguard the future of coral reefs in a changing  
99 climate. Based on comparative analyses of the *Ostreobium* genome with those of other green  
100 algae, we show *Ostreobium*'s innovations in light harvesting antennae and derive insights and  
101 hypotheses about its functions as a coral symbiont, and as an alga living in an extreme  
102 environment.

## 103 **Results and Discussion**

104 We obtained the nuclear genome data of *Ostreobium quekettii* (SAG culture collection  
105 strain 6.99, non-axenic) by assembling the sequenced reads generated by Illumina and Nanopore

106 platforms (Supplementary Table 1). This strain was discovered growing on a culture of a small  
107 tropical red alga and isolated into culture. The strain is nested in a lineage of *Ostreobium*  
108 species found in scleractinian coral<sup>18</sup> and it readily colonizes coral skeleton when that is  
109 provided as a substrate. This clearly shows that the strain is an appropriate representative for  
110 this limestone-burrowing coral-associated genus, despite it being initially isolated from a non-  
111 coral source. *Ostreobium* is known to produce flagellated spores and we think that a spore  
112 residing on the surface of the red alga is the most likely source of the strain.

113 We assembled the nuclear genome of *Ostreobium quekettii* in 2,997 scaffolds (total 150.4  
114 Mb of assembled bases; N50 length 71,393 bp), with an average sequence coverage of 116x for  
115 short-read data. The GC content of the *Ostreobium* nuclear genome is 52.3%, which is higher  
116 than 40.4% of *Caulerpa lentillifera* (the closest relative of *Ostreobium* for which a nuclear  
117 genome has been sequenced), but less than most other green algae (Supplementary Table 2).  
118 The annotation process resulted in 11,735 predicted protein-coding genes, of which ~52% were  
119 supported by RNA sequencing data. We recovered 74.3% of complete core conserved eukaryote  
120 genes (based on BUSCO<sup>19</sup>) in the assembled genome, comparable to the 75.9% in both *C.*  
121 *lentillifera* and *Ulva mutabilis* (Supplementary Fig. 1).

## 122 **Photobiology in a dark place**

123 *Ostreobium* has developed adaptations to the peculiar light environment in which it lives,  
124 but its mechanisms are not well understood<sup>13,14</sup>. Our investigation of the light harvesting  
125 complex (LHC) proteins shows that *Ostreobium* has the highest number of LHC proteins  
126 observed among green algae and *Arabidopsis thaliana* (Fig. 2a, Supplementary Figs 1 and 2).  
127 This expansion of the light harvesting protein arsenal is found in both photosystems, with  
128 duplications of the *Lhca1* and *Lhca6* gene families associated with PSI (Supplementary Fig. 2)  
129 and the presence of both *Lhcp* and *Lhcb* families associated with PSII (Supplementary Fig. 3).

130 Most green algae possess a single *Lhca1* gene, while *Ostreobium* has four (Fig. 2); two  
131 adjacent genes were found on two different scaffolds, suggesting that this gene family expanded  
132 via tandem duplication and translocation (Fig. 2b). The specific amino acid residue as a ligand

133 for Chl in LCH proteins is essential in determining the chromophore organization, which affects  
134 light spectral absorption<sup>20</sup>. The Lhca1 protein of green plants typically uses histidine as the  
135 chlorophyll-binding residue (A5 site in Fig. 2c), but *A. thaliana* mutants use asparagine at the  
136 A5 site, yielding red-shifted absorption spectra<sup>20,21</sup>. Although most green algae use histidine at  
137 the A5 site, the siphonous green algae (*Ostreobium*, *C. lentillifera* and *Bryopsis corticulans*) all  
138 have asparagine (Fig. 2c), hinting at their capability to use far-red wavelengths. Lhca6 is located  
139 in the outer LHC belt, where it forms heterodimers (with Lhca5), and their long C-terminal  
140 loops facilitate interactions between the inner and outer LHC belts<sup>22,23</sup>. The *Lhca6* gene occurs  
141 as a single copy in most microalgae and is absent in the seaweeds *C. lentillifera* and *U.*  
142 *mutabilis*. Remarkably, we recovered six copies of *Lhca6* in *Ostreobium* (Supplementary Fig.  
143 2). This result is supported by transcriptomic evidence for all *Lhca6* genes and different  
144 chromosomal contexts (i.e. flanking genes) on the contigs.

145 In the PSII-associated LHC, *Ostreobium* possesses an unusual combination of both the  
146 Lhcp and major light-harvesting complex (Lhcb) protein families, and has more copies of both  
147 genes than do other green algae (Fig. 2a, Supplementary Fig.3). Lhcb is found in most species  
148 of the green lineage except prasinophytes that have the Lhcp family instead<sup>24</sup>. Only the  
149 streptophyte *Mesostigma viridis* is known to encode proteins of both families (Supplementary  
150 Fig. 3) suggesting that Lhcp and Lhcb were both part of the LHCII antenna system of the green  
151 plant ancestor and the families were differentially lost in different green algal and land plant  
152 lineages<sup>25,26</sup>.

153 Although *Ostreobium* shows a high diversity of LHC genes, it lacks many genes for  
154 photoprotection and photoreceptors. The non-photochemical quenching (NPQ) genes for  
155 LHCSR and PsbS are both absent from *Ostreobium* and *C. lentillifera* genomes (Supplementary  
156 Fig. 3 and Supplementary Table 3). Energy-dependent quenching (qE) was not observed in  
157 other siphonous green algae<sup>27</sup> and our genomic data provide clear evidence that these algae are  
158 not capable of this mechanism. *Ostreobium* also lacks the light-harvesting complex-like (LIL)  
159 genes coding for OHP1 and OHP2 (Supplementary Table 3). While the function of LIL proteins  
160 has not been comprehensively determined, their involvement in response to light stress is

161 known<sup>28</sup>. The loss of genes involved in high-light sensitivity also extends to the chloroplast  
162 genome of *Ostreobium*, which lacks the chloroplast envelope membrane protein gene (*cemA*)  
163 that is needed in *Chlamydomonas* to persist in high-light conditions<sup>29</sup>.

164 *Ostreobium* has fewer known photoreceptors than do most other green algae. We found  
165 three blue light photoreceptor genes: a phototropin (not shown), a plant cryptochrome and a  
166 photolyase/blue-light receptor 2 (PHR2; Supplementary Fig. 4). Phototropins are widespread in  
167 green plants<sup>30</sup>. In *C. reinhardtii*, phototropins are involved in the sexual cycle<sup>31</sup>, phototactic  
168 response, light-dependent expression of LHC proteins, chlorophyll and carotenoid biosynthesis,  
169 and LHCSR3-based non-photochemical quenching<sup>32</sup>. However, the function of phototropin in  
170 *Ostreobium* remains unknown, and we did not recover LHCSR in the genome. Surprisingly,  
171 given the predominance of far-red light in coral skeletons, no genes coding for the red/far-red  
172 phytochromes were found in the *Ostreobium* genome. Although phytochromes are widely found  
173 in the green plant lineage, they have been lost in most of the green algal lineage Chlorophyta<sup>33</sup>,  
174 although not in *Micromonas*, a prasinophyte microalga<sup>34</sup>. Most green algae appear to have no  
175 specific photoreceptors for red light<sup>35</sup>. However, *Chlamydomonas* has an animal-like  
176 cryptochrome that can be activated by either red or blue light<sup>36</sup>. The function of this protein is  
177 related to transcription of genes involved in photosynthesis, pigment biosynthesis, cell cycle  
178 control and circadian clock<sup>37</sup>. This gene is not found in *Ostreobium*, which also lacks the Cry-  
179 DASH-type cryptochromes observed in many other green algae (Supplementary Fig. 4).

180 Functional information for Cry-DASH is still scarce<sup>35</sup>, but this protein is known to be involved  
181 in DNA repair in *Arabidopsis*<sup>38</sup>. *Ostreobium* does have two genes similar to the *Arabidopsis*  
182 putative blue-light receptor protein called PAS/LOV (Uniprot O64511)<sup>39</sup>. The UV resistance  
183 locus8 (UVR8), an important photoreceptor to induce UV-B acclimation, is also absent in  
184 *Ostreobium* as well as *C. lentillifera*. We did not find different types of rhodopsin-like  
185 photoreceptors in *Ostreobium*.

186 Besides the photoprotective LHC genes and photoreceptors, *Ostreobium* and *C.*  
187 *lentillifera* appear to have lost the light-dependent protochlorophyllide oxidoreductase (LPOR),  
188 another important light signaling gene involved in chlorophyll biosynthesis. The reduction of

189 protochlorophyllide to chlorophyllide can be catalysed by either of two non-homologous  
190 enzymes: the nuclear encoded LPOR and the plastid-encoded light-independent (DPOR)  
191 protochlorophyllide oxidoreductase <sup>40</sup>. Most green algae have both systems, and DPOR has  
192 been lost in many eukaryotes <sup>41</sup>. The *Ostreobium* genome provides the first evidence for the loss  
193 of LPOR in any eukaryote, and a screening of *C. lentillifera* also came back negative,  
194 suggesting that the loss may have occurred in the common ancestor of Bryopsidales. Both  
195 genera encode DPOR in their chloroplast genomes <sup>29,42</sup>. Although both enzymes catalyse the  
196 same reaction, they have different features: DPOR is encoded, synthesized and active in the  
197 plastid, and is highly sensitive to oxygen <sup>43</sup>, while the nucleus-encoded LPOR is synthesized in  
198 the cytosol and active in the plastid, and requires light to be activated <sup>44</sup>. DPOR might be an  
199 advantage over LPOR for endolithic photosynthetic organisms because of the low-light, low-  
200 oxygen environments they inhabit <sup>4</sup>.

201       The *Ostreobium* genome clearly reflects its evolutionary trajectory into a peculiar light  
202 habitat, with an unparalleled arsenal of LHC proteins but few known mechanisms to sense the  
203 light or defend itself against excessive light. Some of these genome features are shared with  
204 other Bryopsidales, including the loss of LPOR, qE-type non-photochemical quenching and the  
205 UV resistance locus 8. This suggests that the common ancestor of Bryopsidales may have been  
206 a low-light-adapted organism, possibly an endolithic alga like *Ostreobium* is now, a hypothesis  
207 supported by *Ostreobium* being the sister lineage of all other Bryopsidales <sup>45</sup> and other  
208 bryopsidalean lineages also containing old, largely endolithic families <sup>46</sup>. Bryopsidales  
209 originated in the Neoproterozoic with *Ostreobium* diverging in the early Paleozoic <sup>17,47</sup>. One  
210 could speculate that the bryopsidalean ancestor inhabited a low-light environment, possibly on  
211 the dimly lit seafloor beneath Cryogenian ice sheets. The different lineages emerging from this  
212 ancestor could then have followed different evolutionary trajectories during the onset of  
213 Paleozoic grazing, with the *Ostreobium* lineage fully committing to an endolithic lifestyle while  
214 other bryopsidalean lineages engaged in an evolutionary arms race with grazers to form larger  
215 and chemically defended macroalgae.



216 The unusually large arsenal of LHC proteins appears to be confined to the *Ostreobium*  
217 lineage. The genus is present in a diverse range of light environments, from old oyster shells in  
218 the intertidal, where it experiences full spectrum sunlight, to coral skeleton (far-red enriched)  
219 and in mesophotic habitats (blue light), hinting at its capability to harvest energy from photons  
220 of many different wavelengths<sup>48</sup>. While from the genome data alone, we cannot derive that the  
221 different LHC proteins convey a capability for photosynthesis at different wavelengths, it is  
222 known that different molecular arrangement of LHC proteins and the specific pigments binding  
223 to them have an impact on their spectral properties<sup>49</sup>, which could help *Ostreobium* acclimate to  
224 different light environments.

## 225 **Life in an extreme environment**

226 The endolithic environment, and the coral skeleton, is an extreme environment in many  
227 ways. Oxygen levels vary strongly, from high concentrations caused by photosynthesis during  
228 the day to complete anoxia due to respiration during the night, and this trend is mirrored in  
229 strong diurnal pH fluctuations<sup>4,15</sup>. Reactive oxygen species (ROS) can be produced in high  
230 quantities in these conditions, particularly in the morning when photosynthesis starts<sup>50</sup>.

231 The *Ostreobium* genome exhibits a strong genetic capacity for oxidative stress response,  
232 with ROS scavenging and neutralizing genes present in large numbers compared to other green  
233 algae (Fig. 3). We found six genes coding for superoxide dismutases (SOD; Fig. 3 and  
234 Supplementary Fig. 5), metal-containing enzymes that form the first line of defence to  
235 scavenging ROS by catalysing the very reactive superoxide radical ( $O_2^-$ ) into hydrogen  
236 peroxide ( $H_2O_2$ )<sup>51</sup>. All three categories of SOD are present in *Ostreobium*.

237 Hydrogen peroxide is commonly processed by catalase (CAT), of which *Ostreobium* has  
238 eight copies while most other green algae have one or two (Fig. 3), or none in the case of  
239 prasinophytes. Seven of the *Ostreobium* catalases formed a unique lineage in our phylogenetic  
240 analysis (Supplementary Fig. 5) and are found in tandem on three different scaffolds, indicating  
241 diversification of this gene in the *Ostreobium* lineage. Hydrogen peroxide can also be processed  
242 through the glutathione-ascorbate cycle, a metabolic pathway neutralizing hydrogen peroxide

243 through successive oxidations and reductions of ascorbate, glutathione, and NADPH<sup>51</sup> (Fig. 3).  
244 *Ostreobium* featured high copy numbers of the enzymes to quickly purify ascorbate, with eight  
245 copies of ascorbate peroxidase (APX) and seven monodehydroascorbate reductases (MDHAR)  
246 (Supplementary Fig. 5). The glutathione-ascorbate cycle is important to keep ascorbate free for  
247 H<sub>2</sub>O<sub>2</sub> scavenging (Fig. 3).

248 Most algae live in environments with higher oxygen concentrations and can produce  
249 energy via the respiratory electron transport chain. In the coral skeleton, however, any oxygen  
250 produced through photosynthesis is completely consumed by respiration within an hour of the  
251 onset of darkness<sup>15</sup>, and the environment is anoxic for long periods of time, hence we expect  
252 *Ostreobium* to use other electron acceptors to produce ATP and re-oxidize NAD(P)H and  
253 FADH<sub>2</sub>. *Ostreobium* possesses the enzymes required to produce succinate, lactate, formate,  
254 acetate, ethanol, alanine, and glycerol (Supplementary Fig. 6), but lacks H<sub>2</sub> and acetate  
255 production from acetyl-CoA (PAT1/PAT2 and ACK1/ACK2). Several fermentation-related  
256 genes are present in multiple copies (Supplementary Table 4), including two lactate  
257 dehydrogenases, four tandem copies of ALDH (aldehyde dehydrogenase), and eight copies of  
258 malate dehydrogenase, implying that acetate, succinate and lactate are the preferred  
259 fermentation products in *Ostreobium*. We did not find the gene for pyruvate decarboxylase,  
260 which catalyses the production of acetaldehyde, the substrate of ALDH. This gene is also absent  
261 in *C. lentillifera*, *U. mutabilis* and prasinophytes, indicating it has been lost multiple times, and  
262 it is reasonable to assume that another yet uncharacterised enzyme may catalyse this reaction in  
263 these algae.

264 A comparison of the number of genes having particular InterPro annotations between  
265 *Ostreobium* and *Caulerpa* showed a large number of depleted IPR terms in *Ostreobium*, which  
266 can be attributed to an enrichment in the *Caulerpa* genome, as counts in *Ostreobium* are  
267 comparable to those of other green algae. However, genes coding peptidases were strongly  
268 enriched in *Ostreobium* (e.g. Peptidase S1, PA clan – 179 genes; Serine-proteases trypsin  
269 domain – 142 genes; Supplementary Fig. 7). About 40% of the genes are predicted to be  
270 secreted (signal peptide), and 25% are membrane bound, compared to 31% and 12% (of total 57

271 genes) respectively in *C. lentillifera*, suggesting an expanded potential for external protein  
272 degradation in *Ostreobium*. It is known that *Ostreobium* can penetrate the organic-inorganic  
273 composite material of the black pearl oyster nacreous layer<sup>52</sup>, and the combination of  
274 proteolytic enzyme proliferation and its growth at excessively low light intensities lends support  
275 to the hypothesis that this alga could complement its energy needs by feeding on the organic  
276 matrix of the coral skeleton and shells.

## 277 **The coral holobiont**

278 *Ostreobium* plays a number of important roles in the coral holobiont, particularly during  
279 periods of coral bleaching<sup>4</sup>, but current knowledge is far from complete and the genome can  
280 help define hypotheses of how the species may interact with other holobiont members.

### 281 *Molecular mechanism of CaCO<sub>3</sub> dissolution*

282 *Ostreobium* is the main agent of microbial bioerosion of the coral skeleton. While the  
283 molecular mechanism behind this phenomenon is not yet known<sup>53</sup>, the *Ostreobium* genome  
284 reveals the molecular toolkit available for this process and allows us to make conjectures about  
285 how bioerosion by *Ostreobium* might occur (Fig. 4a). A working model for microbial carbonate  
286 excavation was first described for cyanobacteria, involving passive uptake of Ca<sup>2+</sup> at the boring  
287 front, decreasing the ion concentration in the extracellular area below calcite saturation levels  
288 and leading to dissolution of adjacent calcium carbonate<sup>54</sup>. Imported Ca<sup>2+</sup> is transported along  
289 the cyanobacterial filament and excreted away from the growing tip, likely by P-type Ca<sup>2+</sup>-  
290 ATPases that pair transport of Ca<sup>2+</sup> with counter transport of protons<sup>54</sup>. Whereas the Ca<sup>2+</sup>-  
291 ATPase mechanism appears to be somewhat widespread among endolithic cyanobacteria, it is  
292 unclear whether this mechanism extends to microboring algae like *Ostreobium*<sup>55</sup>.

293 *Ostreobium* has an expanded repertoire (33 genes) of calcium transporters  
294 (Supplementary Table 5), including 18 voltage-dependent calcium channels and several  
295 transient receptor potential transporters, two-pore channels and calcium-transporting ATPases.  
296 Calcium uptake, possibly combined with acidification via a Ca<sup>2+</sup>/H<sup>+</sup> transporter would promote

297 decalcification (Fig. 4a), allowing *Ostreobium* to burrow into the coral skeleton. Calcium  
298 toxicity can be avoided either by accumulation in a vacuole and/or posterior transport out of the  
299 cell. In addition to calcium transport, bicarbonate uptake could also play a role in the burrowing  
300 mechanism. *Ostreobium* carries two orthologs of the CIA8 transporter responsible for  
301 bicarbonate transport in *C. reinhardtii*<sup>56</sup>, and the imported bicarbonate may be further  
302 transported into the chloroplast to be fixed. *Ostreobium* has a carbonic anhydrase predicted to  
303 be targeted outside the cell that may further assist the decalcification process. In natural  
304 communities dominated by *Ostreobium*, CaCO<sub>3</sub> dissolution was higher at night, suggesting that  
305 *Ostreobium* takes advantage of the lower external pH at night<sup>53</sup>.

306         Whilst the *Ostreobium* genome does not provide confirmation of a specific mechanism  
307 for CaCO<sub>3</sub> dissolution, it lends support to a growing hypothesis of how bioerosion might work  
308 in *Ostreobium*, likely bearing similarities to the process described in cyanobacteria. A detailed  
309 characterisation of this dissolution process should be a priority, as *Ostreobium* is responsible for  
310 ca. 30-90% of microbial dissolution of skeletal CaCO<sub>3</sub>. Higher temperatures and lower pH boost  
311 this activity, suggesting that this yet unknown process will lead to major reef deterioration in  
312 future ocean conditions<sup>53</sup>.

### 313 *Interactions with other holobiont members*

314         As far as holobiont functioning goes, interactions between the coral animal and its algal  
315 and bacterial symbionts are best understood, and interactions between the Symbiodiniaceae and  
316 bacteria are just starting to come into focus<sup>57</sup>, but little is known about interactions involving  
317 *Ostreobium*. It is well known that several algae lack genes related to biosynthetic pathways of,  
318 and are auxotrophic for, certain nutrients. The *Ostreobium* genome shows that the metabolic  
319 pathways involved in the production of vitamins B1, B2, B6 and B9 are complete, but it lacks  
320 the BioA and LysA genes involved in biotin and lysine synthesis. These genes were found in all  
321 other green algae used for comparison, suggesting that this gene loss is specific to *Ostreobium*.  
322 It is likely that the alga obtains these nutrients from bacteria, and the dependency on external  
323 lysine may also explain the enrichment of extracellular proteases encoded in the *Ostreobium*

324 genome. Many algae are auxotrophic for vitamin B12 (cobalamin), a cofactor involved in the  
325 synthesis of methionine, instead obtaining this compound from associated bacteria<sup>58</sup>. The  
326 *Ostreobium* genome encodes two gene copies of a B12-independent methionine synthase  
327 (METE) in addition to the B12-dependent version (METH,<sup>59</sup>), so while the alga most likely  
328 does not strictly require B12 for growth, the presence of METH suggests that it uses B12  
329 provided by other holobiont members. Corals are also auxotrophic for several vitamins and  
330 amino acids that are produced by holobiont members<sup>2</sup> (Fig. 4b).

331 The nature of metabolic exchanges between *Ostreobium* and the coral animal is an open  
332 question in holobiont research, and a potentially critical one, as coral bleaching and subsequent  
333 blooming of the endolithic *Ostreobium* algae become increasingly common due to ocean  
334 warming. Typical algal-animal metabolic exchanges include nitrogen and CO<sub>2</sub> provision by the  
335 animal to the alga and carbohydrate provision to the animal by the alga<sup>60,61</sup>. Coral polyps are  
336 known to secrete nitrogen in the form of ammonia. An expanded repertoire of ammonia  
337 transporters was identified in *Ostreobium* (Supplementary Table 5), potentially reflecting an  
338 adaptation to increase and diversify ammonia uptake in the alga. This observation is also in line  
339 with the presence of diazotrophic bacteria facilitating the conversion of N<sub>2</sub> into ammonia in  
340 marine limestones (including coral skeletons) and ammonia being the most abundant form of  
341 inorganic nitrogen in skeletal pore waters<sup>62</sup>, and adds to the evidence for the roles that  
342 endolithic organisms play in the holobiont N cycle. During carbon cycling in the holobiont,  
343 glucose has been postulated as one of the main carbohydrates exchanged between  
344 Symbiodiniaceae and corals<sup>60</sup>. While carbon compounds fixed by endoliths are known to be  
345 transferred to the coral animal and subsequently assimilated, neither the exact transferred  
346 molecules nor the molecular mechanisms involved in their translocation have been  
347 characterised, but the *Ostreobium* genome encodes two genes coding for H<sup>+</sup>-glucose  
348 transporters that might be involved in this process.

349 *Probing changes in holobiont processes*

350 While these investigations of the *Ostreobium* genome allowed us to evaluate hypotheses  
351 about interactions within the holobiont, gaining deeper insight will require approaches that  
352 study multiple partners simultaneously. As a first step towards understanding the molecular  
353 mechanisms in the coral holobiont during coral bleaching, we screened transcriptomes of  
354 healthy and bleached coral holobionts. We exposed fragments of the coral *Orbicella faveolata*  
355 to elevated temperatures leading to bleaching, followed by re-acclimation of the bleached  
356 samples to ambient temperatures (post-bleaching condition), during which the corals remained  
357 bleached and an *Ostreobium* bloom occurred. Total metatranscriptomes (including coral tissue  
358 and skeleton) were generated for healthy control samples that were kept under ambient  
359 temperature (no bleaching), and the bleached samples.

360 From the KEGG annotation of the assembled transcriptome, it is clear that the number of  
361 transcripts coding for the *Ostreobium* photosynthetic apparatus exceeds that of Symbiodiniaceae  
362 (Supplementary Fig. 8), in line with the expanded set of photosynthetic genes (particularly  
363 LHC) observed in the *Ostreobium* genome. While the metatranscriptome sequencing depth was  
364 not designed to track up- and down-regulation of individual genes, it was clear that expression  
365 of the photosynthesis genes *psbA* and *rbcL* from Symbiodiniaceae was drastically lower in  
366 bleached samples while expression of these genes in *Ostreobium* increased (Supplementary Fig.  
367 9a), supporting the notion that *Ostreobium* becomes a dominant agent of photosynthesis in the  
368 bleached holobiont. The bleached state increases the light available to *Ostreobium*, which likely  
369 leads to the need for higher repair rates of PSII protein D1 (encoded by *psbA*) and higher rates  
370 of carbon fixation (facilitated by RuBisCO, encoded by *rbcL*). The expression of these genes by  
371 Symbiodiniaceae in the bleached samples, albeit at low levels, suggests that some of these  
372 endosymbionts have remained or that some re-colonisation has occurred during the post-  
373 bleaching period.

374 We detected multiple haplotypes of *psbA* (Supplementary Fig. 9b), indicating that several  
375 strains of *Ostreobium* were present in the *O. faveolata* skeletons<sup>63</sup>. While expression levels for

376 these haplotypes tended to increase, some did not change significantly while others differed by  
377 an order of magnitude or more. These differences suggest that *Ostreobium* strains may differ  
378 physiologically or change in relative abundance during the experiment. Such differences in the  
379 microbiome – whether *Ostreobium* strains or other holobiont members – may result in high  
380 variability among coral samples in experimental work. Indeed, we found considerable  
381 differences in expression levels between samples within conditions, suggesting that future  
382 metatranscriptome experiments should be planned to use generous replication.  
383 The metatranscriptomes also provide some hints as to where *Ostreobium* may source its needs  
384 for vitamin B12 and biotin. We detected 35 bacterial transcripts from the vitamin B12 pathway,  
385 including several Proteobacteria (e.g. Rhodobacteraceae), Bacteroidetes (e.g. Flavobacteriaceae)  
386 and Cyanobacteria that are known to be abundant in the coral skeleton <sup>46</sup>. Seven bacterial  
387 transcripts associated with the biotin pathway were found, with taxonomic affinities to  
388 Actinobacteria, Cyanobacteria and Proteobacteria (e.g. Endozoicomonadaceae,  
389 Rhodospirillaceae). This result narrows down the potential mutualistic relationships between  
390 bacteria and *Ostreobium* in the holobiont (Fig. 4b).

## 391 **Conclusion**

392 The complexity of the coral holobiont presents an interesting challenge to reconstruct a  
393 comprehensive model of metabolic exchanges and other interactions among its component  
394 organisms. Recent progress in building such models from genomes of coral and tissue-  
395 associated prokaryotes <sup>2</sup> has not been mirrored in the skeleton. Our results allowed refining  
396 hypotheses from previous physiological work, illuminating the biology of skeletal inhabitants  
397 and their interactions. Despite this progress, many questions remain about the exact mechanisms  
398 at play, their immediate physiological effects on the partners and longer-term ecological  
399 consequences. One challenge in this area of research is that the roles and impacts of *Ostreobium*  
400 vary in time. In terms of photosynthesis, *Ostreobium* makes a minor contribution to holobiont  
401 photosynthesis in healthy corals <sup>10</sup>, but our results show that *Ostreobium* blooms during

402 bleaching cause a shift of expression levels between microbiome members. Specifically,  
403 *Ostreobium* takes over the major role of photosynthesis from the Symbiodiniaceae now lost  
404 from the holobiont. These changes likely have implications flowing through the entire  
405 microbiome interaction network, but our knowledge of this is in its infancy. From a  
406 physiological viewpoint, the question remains as to what extent the *Ostreobium* bloom is simply  
407 an opportunistic response, and whether it is actually leading to a beneficial metabolite exchange  
408 with the coral host. Similarly, the fitness costs/benefits of *Ostreobium* in the skeleton need to be  
409 evaluated across the entire coral life cycle and might differ drastically between the period of  
410 early skeleton formation following larval settlement and at later stages. The fact that there are  
411 >80 different species-level operational taxonomic units in *Ostreobium*, and virtually nothing is  
412 known their physiological features, complicates the matter further<sup>46,63</sup>. Genome-scale data from  
413 *Ostreobium* and other interacting partners critically link these physiological features to  
414 underlying molecular mechanisms. The results and datasets generated in this study provide a  
415 foundational reference for future research into the biology of this key holobiont member, and  
416 the intricate role that *Ostreobium* plays in coral biology. This will be of particular importance in  
417 the light of global climate change, as the increased frequency of bleaching and lower pH will  
418 boost *Ostreobium* populations and carbonate dissolution rates.

## 419 **Methods**

### 420 **Culturing and nucleic acid extraction**

421 *Ostreobium quekettii* (SAG culture collection strain 6.99, non-axenic) was cultured in F/2  
422 media on a 14H - 8H light/dark cycle at ~19 degrees celsius. Total DNA was extracted using a  
423 modified cetyl trimethylammonium bromide (CTAB) method described in Cremen and  
424 collaborators<sup>64</sup>. Total RNA was extracted using Plant RNA reagent (Thermofisher, Waltham,  
425 MA, USA).



## 426 **Illumina sequencing and assembly**

427 Total DNA was sequenced using Illumina sequencing technology (HiSeq2000, 150 bp  
428 paired-end reads, ~40 GB data) (Supplementary Table 1), at Novogene, Beijing. Reads were  
429 trimmed with Cutadapt v1.12<sup>65</sup> using the parameters -e 0.1 -q 10 -O 1 -a AGATCGGAAGAGC.  
430 To remove plDNA, mtDNA and bacterial sequences prior to assembly all reads were mapped  
431 against the previously published *Ostreobium* chloroplast and mitochondrial sequences and a  
432 database of bacterial genomes, using BWA with the bwa-mem algorithm at default settings<sup>66</sup>.  
433 The bacterial database was constructed from 5460 prokaryotic representative genomes  
434 ([https://www.ncbi.nlm.nih.gov/refseq/about/prokaryotes/#representative\\_genomes](https://www.ncbi.nlm.nih.gov/refseq/about/prokaryotes/#representative_genomes)) downloaded  
435 from NCBI using the tool ncbi-genome-download ([https://github.com/kblin/ncbi-genome-](https://github.com/kblin/ncbi-genome-download)  
436 [download](https://github.com/kblin/ncbi-genome-download)). If one or both pair-end reads mapped to any database sequence, both reads were  
437 removed from the full Illumina dataset using the BBtools ‘filterbyname.sh’ program  
438 (<https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/>). The resulting dataset was  
439 assembled using Spades v3.12.0 with the --careful option<sup>67</sup>. Contigs produced by Spades were  
440 further filtered to identify any remaining bacterial or fungal sequences by BLAST searches of  
441 open reading frames against the nr database.

442 For RNA sequencing, a strand-specific 100 bp paired-end library was constructed and  
443 sequenced using Illumina HiSeq 2500 (ENA study accession number PRJEB35267). Quality  
444 filtering of reads was performed using Trimmomatic v0.39<sup>68</sup> with the following settings:  
445 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:20. Transcriptome data were assembled  
446 using Trinity v2.8.3<sup>69</sup> in both genome-guided mode and de novo mode.

## 447 **Nanopore sequencing and hybrid assembly**

448 Total DNA was extracted as above and sequenced using nanopore sequencing technology  
449 (MinION, Oxford Nanopore Technologies), producing 1,889,814 reads and ~9.5 GB data. To  
450 identify sequences originating from bacteria, all reads were used as blastn queries (megablast

451 algorithm) against the prokaryotic representative genome dataset. Reads with a hit alignment  
452 >1000 bp long and an e-value < 1e-100 were removed from the nanopore dataset.

453 A hybrid (combined long-read and short-read) genome assembly was performed with  
454 MaSuRCA v3.2.7<sup>70</sup>, using the filtered Illumina reads as short-read input. For long-read input,  
455 the nanopore reads were complemented with the contigs produced from the Spades assembly of  
456 Illumina data and treated as ‘pseudo-nanopore’ reads.

## 457 **Ab initio prediction of protein-coding genes**

458 We adapted the workflow from<sup>71</sup> for ab initio prediction of protein-coding genes in the  
459 *Ostreobium* genome assembly. Repetitive elements in the genome assembly were first predicted  
460 de novo using RepeatModeler v1.0.11 (<http://www.repeatmasker.org/RepeatModeler/>). These  
461 repeats were combined with known repeats in the RepeatMasker database (release 20171107) to  
462 generate a customized repeat library. All repetitive elements in the assembled genome scaffolds  
463 were then masked using RepeatMasker 4.0.7 (<http://www.repeatmasker.org/>) based on the  
464 customized repeat library, before they were subjected to prediction of genes.

465 The assembled transcripts were used as transcriptome evidence, and vector sequences  
466 were removed using SeqClean<sup>72</sup> based on UniVec (build 10) database. PASA pipeline v2.3.3<sup>73</sup>  
467 and TransDecoder v5.2.0<sup>74</sup> were first used to predict protein-coding genes (and the associated  
468 protein sequences) from the vector-trimmed transcriptome assemblies (hereinafter transcript-  
469 based genes). The predicted protein sequences from multi-exon transcript-based genes with  
470 complete 5' and 3'-ends were searched (BLASTp, E ≤ 10-20) against RefSeq proteins (release  
471 88). Genes with significant BLASTp hits (>80% query coverage and >80% subject coverage)  
472 were retained. Transposable elements were identified using HHblits<sup>75</sup> and Transposon-PSI  
473 (<http://transposonpsi.sourceforge.net/>), searching against the JAMg transposon database  
474 (<https://github.com/genomecuration/JAMg>). Proteins putatively identified as transposable  
475 elements were removed. Those remaining were clustered using CD-HITS (ID=75%)<sup>76</sup> to yield a  
476 non-redundant protein set, and the associated transcript-based genes were kept. These genes  
477 were further processed by the Prepare\_golden\_genes\_for\_predictors.pl script from the JAMg

478 package (<https://github.com/genomecuration/JAMg>). This step yielded a set of high-quality  
479 “golden” genes, which were used as a training set for gene prediction using AUGUSTUS <sup>77</sup> and  
480 SNAP <sup>78</sup>. We also employed GeneMark-ES v4.38 <sup>79</sup> to generate prediction from the genome  
481 scaffolds, and MAKER protein2genome v2.31.10 <sup>80</sup> to make predictions based on homology  
482 evidence to SwissProt proteins (downloaded 27 June 2018).

483 Subsequently, all genes predicted using GeneMark-ES, MAKER, PASA, SNAP and  
484 AUGUSTUS were integrated into a combined set using EvidenceModeler v1.1.1 <sup>81</sup>, following a  
485 weighting scheme of GeneMark-ES 2, MAKER 8, PASA 10, SNAP 2, AUGUSTUS 6. The  
486 resulting genes from the EvidenceModeler prediction were retained if they were constructed  
487 using evidence from PASA, or using two or more other prediction methods.

488 Functional information of translated predicted genes was retrieved using search BLASTP  
489 against UniProt databases (Swiss-Prot and TrEMBL), KEGG’s annotation tool BlastKOALA <sup>82</sup>.  
490 Gene models of the genome dataset were annotated using InterProScan 5.39 <sup>83</sup> using InterPro  
491 (version 77.0 databases) of Pfam (32.0), SUPERFAMILY (1.75) and TIGRFAMs (15.0) <sup>84</sup>. The  
492 *Ostreobium* genomic data is deposited in <https://cloudstor.aarnet.edu.au/plus/s/X9xS30hE7>.

## 493 **Orthogroups, phylogenetic analysis and BUSCO analysis**

494 For comparative genomic analyses, we built a dataset containing genomes and  
495 annotations of 20 green algae and two land plants (Supplementary Table 4). We used the  
496 OrthoFinder 2.3.7 <sup>85</sup> pipeline (default parameters) to cluster the potential orthologous protein  
497 families. Enrichment and depletion of domains in *Ostreobium* versus *Caulerpa lentillifera* were  
498 identified using Fisher's exact tests with a false discovery rate correction (Benjamini-Hochberg  
499 FDR method) of 0.05. All statistical tests were carried out in R <sup>86</sup>. Subcellular localization of  
500 sequences of interest was performed using PSORT <sup>87</sup>, using the WoLF PSORT web server  
501 (<https://wolfpsort.hgc.jp/>) with the ‘plant’ option, and PredAlgo <sup>88</sup>, using default settings.

502 We performed phylogenetic analyses for protein families relevant for photobiology and  
503 oxidative stress response, based on the orthogroups obtained from the OrthoFinder analysis  
504 described above. All phylogenetic trees were reconstructed using IQTREE 1.6.12 with the built-

505 in model selection function, and branch support estimated using ultrafast bootstrap with 1,000  
506 bootstrap replicates<sup>89</sup>. To identify the light-harvesting protein families associated with PSI  
507 (Lhca), we also included Lhca protein sequences from *Bryopsis corticulans*<sup>23</sup> and  
508 *Chlamydomonas reinhardtii*<sup>22</sup>.

509 We ran BUSCO v3.1.0<sup>19</sup> on the *Ostreobium* genome using the Eukaryota dataset. This  
510 analysis identifies complete, duplicated, fragmented and missing genes that are expected to be  
511 present in a set of single-copy genes in the dataset. We ran identical BUSCO analyses on the *C.*  
512 *lentillifera*, *Ulva mutabilis* and *C. reinhardtii* genomes to allow direct comparison.

### 513 **Metatranscriptome analysis of healthy and bleached corals**

#### 514 *Experiment*

515 *Orbicella faveolata* fragments (4cm<sup>2</sup>) from three different colonies were collected in  
516 Petempiche Puerto Morelos, Quintana Roo, Mexico (N 20° 54'17.0", W 86° 50'11.9") at a  
517 depth between eight and nine meters in June 2013 (Permit registration MX-HR-010-MEX, Folio  
518 036). The coral skeleton and live tissue were collected using a hammer and chisel and were  
519 transported in seawater to perform the experiment at the Instituto de Ciencias del Mar y  
520 Limnología, UNAM. Three fragments were placed in each control and “experimental” tank at  
521 ~28°C. Following 18 days of acclimation, heaters were turned on in the treatment tank to reach  
522 ~32°C. After 5 days of severe heat exposure, bleached corals were moved back into the control  
523 tank to recover at 28°C, where they remained for 38 days (post-bleaching period). Post-bleached  
524 and controlled coral fragments were flash frozen and preserved in liquid nitrogen.

#### 525 *RNA library preparation and sequencing and data availability*

526 Coral fragments were ground to a fine powder in liquid nitrogen. Total RNA was  
527 extracted using the mirVana miRNA Isolation Kit (Life Technologies) since this kit resulted in  
528 higher coral holobiont RNA yield and quality. RNA was purified and concentrated using the  
529 RNA Clean and Concentrator kit (Zymo Research, Irvine, USA). RNA quantification was

530 assessed on a Nanodrop and Qubit using 2.0 RNA Broad Range Assay Kit (Invitrogen). Quality  
531 was verified using the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, USA).  
532 Total RNA samples were sent for metatranscriptome sequencing to the US Department of  
533 Energy's Joint Genome Institute (JGI), California. Samples were depleted in ribosomal RNA  
534 and enriched in mRNA from whole holobionts employing the RiboZero kits (Epicenter).  
535 Following the RiboZero protocol, mRNA was converted to cDNA and amplified. The libraries  
536 were sequenced on the Illumina HiSeq 2000 platform using 2 X 151 bp overlapping paired-end  
537 reads. Raw and filtered metatranscriptome sequence data, statistics and quality sequencing  
538 reports for the experiment are available at the US Department of Energy Joint Genome Institute  
539 (JGI)'s genome portal (<https://genome.jgi.doe.gov/portal/>) with accession codes 1086604,  
540 1086606, 1086608, 1086610, 1086612, 1086614, Community Sequencing Project No. 1622).

#### 541 *Metatranscriptome analysis*

542 The raw reads were quality-trimmed to Q10, adapter-trimmed and filtered for process  
543 artifacts using BBDuk<sup>90</sup>. Ribosomal RNA reads were removed by mapping against a trimmed  
544 version of the Silva database using BBmap (<http://sourceforge.net/projects/bbmap>). To generate  
545 a de novo reference metatranscriptome, cleaned reads from all samples per species (replicates  
546 from control and treatment fragments) were pooled and assembled using Trinity version 2.1.1<sup>74</sup>.

547 We performed a BLASTn search to identify and separate the different members of the  
548 holobiont using a local database. This database was built with the *Ostreobium* genome, the sea  
549 anemones *Nematostella vectensis*<sup>91</sup> and *Exaiptasia diaphana* (syn. *Aiptasia pallida*)<sup>92</sup>, the corals  
550 *Acropora digitifera*<sup>93</sup> and *Orbicella faveolata*<sup>94</sup>, and the Symbiodiniaceae genomes *Breviolum*  
551 *minutum*<sup>95</sup>, *Fugacium kawagutii*<sup>1</sup> and *Symbiodinium microadriaticum*<sup>96</sup>, as well as several  
552 *Breviolum* spp. transcriptomes<sup>97</sup>.

553 Kallisto<sup>98</sup> was used to perform a pseudoalignment and quantify transcript abundances,  
554 using the *Ostreobium* contigs derived from the metatranscriptome assembly as a reference. A  
555 comparison of counts per million, a correlation matrix and principal component analysis among  
556 samples was performed for a quality check of the replicates per species. Expression analysis

557 was performed using the DESeq2 software<sup>99</sup>. Differential Expressed Genes (DEGs) were  
558 defined by using a cutoff threshold of False Discovery Rate FDR <0.001 and log fold change of  
559 2. Enzyme Commission numbers (EC) were retrieved from the Kyoto Encyclopedia of Genes  
560 and Genomes (KEGG) database using MEGAN5<sup>100</sup> for genes from each holobiont member (i.e.  
561 host, Symbiodiniaceae and *Ostreobium*).  
562

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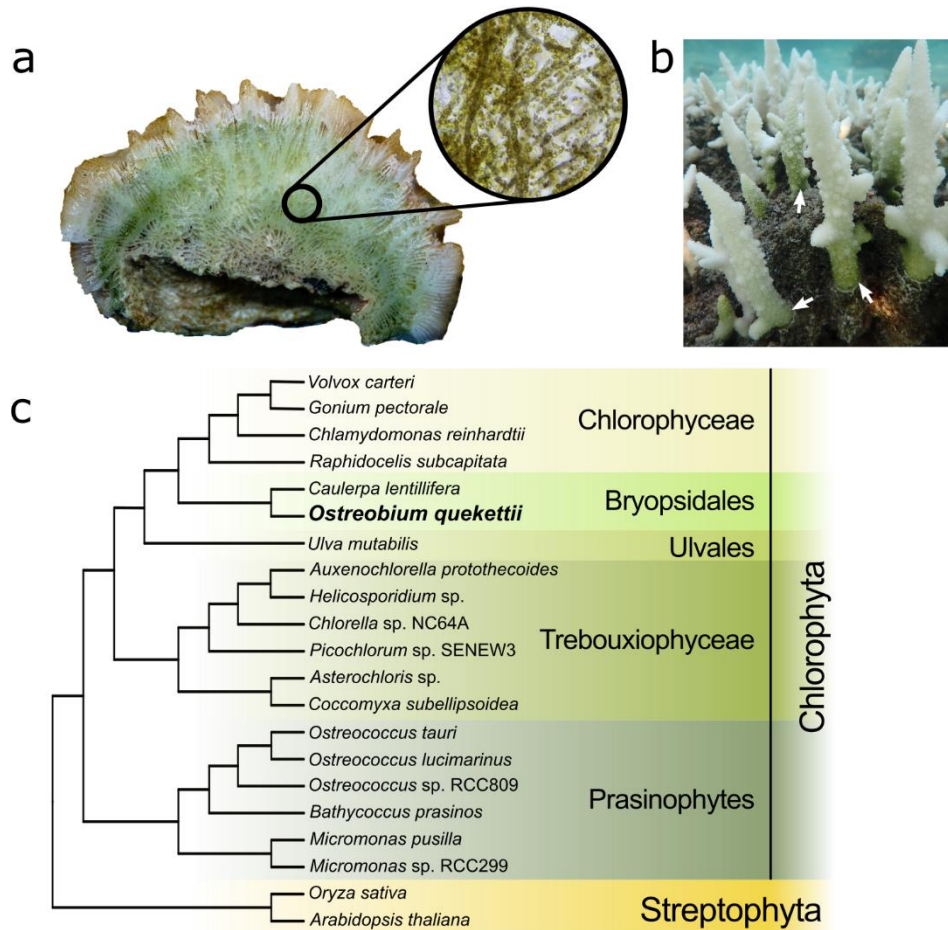


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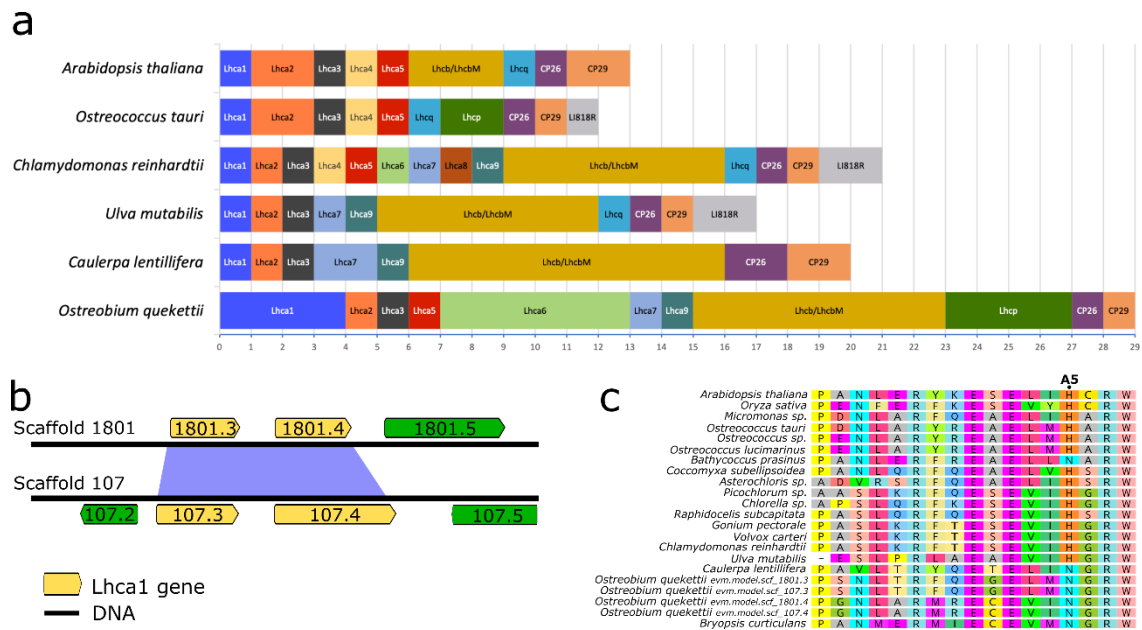
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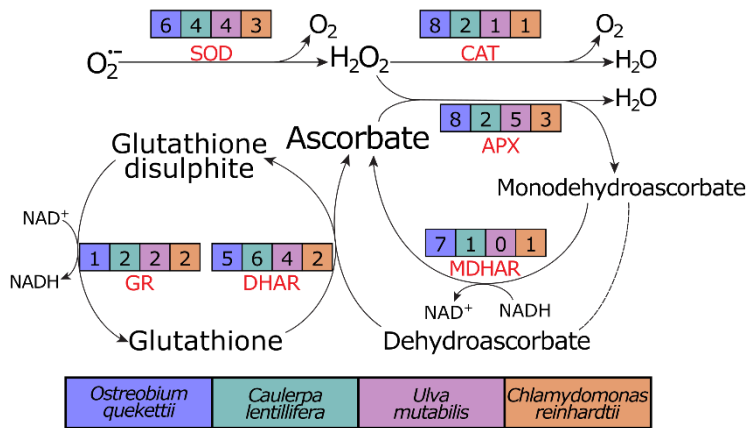
## Figures



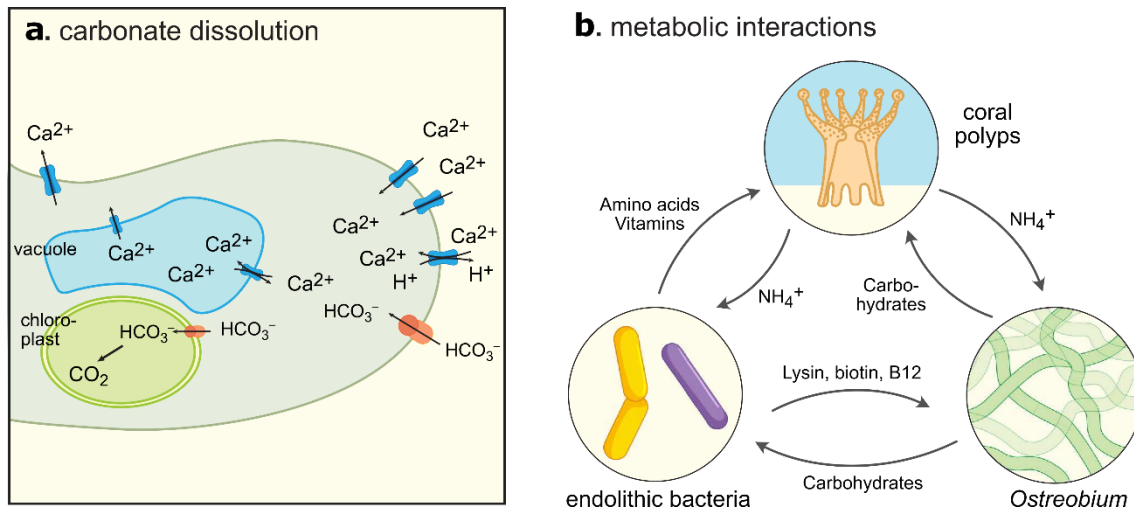
**Figure 1.** (a) Cross-section of *Paragoniastrea australensis* coral showing *Ostreobium* that inhabits the skeleton. The inset shows *Ostreobium* filaments after skeletal decalcification. (b) Bleached coral with evident *Ostreobium* bloom (indicated by white arrows). Photograph by Alexander Fordyce. (c) Phylogenetic tree of Viridiplantae (Streptophyta + Chlorophyta) showing the position of *Ostreobium quekettii*.



**Figure 2.** (a) Number of different light harvesting complex (LHC) proteins in *Ostreobium*, other green algae and *Arabidopsis thaliana*. (b) Synteny of *Lhca1* copies in the *Ostreobium* genome. (c) Amino acid sequence comparison between *Lhca1* proteins, showing asparagine (N) at the chlorophyll-binding residue A5 in *Ostreobium*.



**Figure 3.** Simplified oxidative response pathway comparing the number of genes for enzymes found in the genomes from *Ostreobium* and some other green algae. Compared with *C. lentillifera*, *U. mutabilis* and *C. reinhardtii*, *Ostreobium* does have more copies of genes related to quick response to neutralize ROS, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and monodehydroascorbate reductase (MDHAR). DHAR, dehydroascorbate reductase. GR, glutathione reductase.



**Figure 4.** Roles of *Ostreobium* in the coral holobiont. **(a)** Potential mechanisms available to *Ostreobium* for excavation of the  $\text{CaCO}_3$  skeleton of corals near the growing tip of *Ostreobium* filament. **(b)** Possible interactions between members of the holobiont derived from genome sequence data.