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Every refuge has its price: *Ostreobium* as a model for understanding how algae can live in rock and stay in business

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Abstract

Ostreobium is a siphonous green alga in the Bryopsidales (Chlorophyta) that burrows into calcium carbonate ($CaCO_3$) substrates. In this habitat, it lives under environmental conditions unusual for an alga (i.e., low light and low oxygen) and it is a major agent of carbonate reef bioerosion. In coral skeletons, *Ostreobium* can form conspicuous green bands recognizable by the naked eye and it is thought to contribute to the coral's nutritional needs. With coral reefs in global decline, there is a renewed focus on understanding *Ostreobium* biology and its roles in the coral holobiont. This review summarises knowledge on *Ostreobium*'s morphological structure, biodiversity and evolution, photosynthesis, mechanism of bioerosion and its role as a member of the coral holobiont. We discuss the resources available to study *Ostreobium* biology, lay out some of the uncharted territories in *Ostreobium* biology and offer perspectives for future research.

Keywords: O*streobium*; Endolith; Siphonous green algae; Bioerosion; Corals; Low-light photosynthesis

Highlights

1. *Ostreobium* is a siphonous green alga that lives as an endolith, burrowing microscopic galleries and forming conspicuous green bands in the coral skeleton.
2. *Ostreobium* photosynthesizes in extreme low light conditions and can utilize near-infrared light, which is unusual for a green alga.
3. Photosynthates produced by *Ostreobium* could potentially benefit coral host during stress-induced coral bleaching.
4. *Ostreobium* biodiversity is underestimated and its biology largely remains unexplored owing to difficulties in culturing.
5. Draft genome of *Ostreobium* shed light on its genomic adaptations to the low-light environment but molecular mechanisms of photobiology and calcium carbonate dissolution remain largely unexplored.

1. Introduction

Ostreobium is a genus of siphonous green algae in the order Bryopsidales (Chlorophyta). It tends to live in extreme habitats (for an alga), inside of calcium carbonate ($CaCO_3$) rocks at very low light levels, including the deeper end of the euphotic zone [1–3]. As an endolithic organism, *Ostreobium* can actively burrow its way through the rock substrate that it colonizes. *Ostreobium* has been identified from all sorts of marine carbonate substrates, including limestone reef rocks, bivalve shells and the skeleton of live corals [4,5]. *Ostreobium* is a major agent of reef bioerosion [6].

In its natural habitat, *Ostreobium* can be recognised as grass green bands in the $CaCO_3$ structures it inhabits, often exhibiting clearly defined bands in the coral skeleton (Fig. 1A, B) or more diffuse green areas in other reef carbonates. Like other members in the green algal order Bryopsidales, *Ostreobium* is unicellular and multinucleate, with the giant tubular (siphonous) cell branching to form a dense network when growing in $CaCO_3$ or a diffuse mass of filaments in free culture (Fig. 1D, E; [7–9]).

Numerous roles are documented for *Ostreobium* in coral, including exchange of nitrogen, carbon, and other nutrients [10–12]. *Ostreobium* can supply photosynthates to the coral during coral bleaching when symbiotic dinoflagellates of the family Symbiodiniaceae have been expelled from the coral host (Fig. 1C), but whether such carbon translocation facilitates coral recovery or survival needs further research [13–15]. A recent study has also explored the role of *Ostreobium* in providing partial protection to other symbionts from high light stress and promoting coral recovery after coral bleaching [16].

Ostreobium lives in an extremely low-light habitat. The mechanisms by which it manages to thrive in these conditions remain largely unknown, and many questions about the broader consequences of *Ostreobium*'s presence and activity on its natural habitats remain unanswered.

This paper reviews our current knowledge of *Ostreobium* and discusses its potential as a model organism. We summarize its biodiversity and evolution, then describe organismal and data resources, and discuss research opportunities and challenges. We identify three promising research themes using *Ostreobium* as a model system: low-light photosynthesis, endolithic ecophysiology and coral-algal interactions. Throughout, we highlight knowledge gaps and make recommendations for future studies.

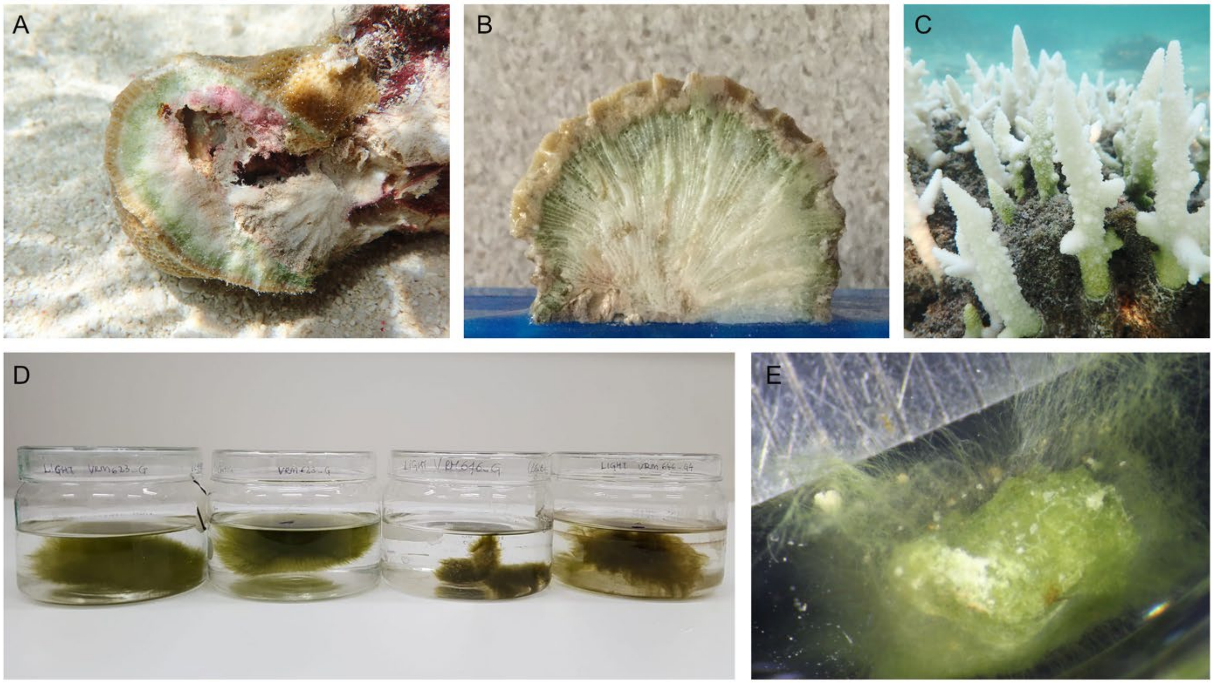


Figure 1. *Ostreobium in situ* and in culture. (A) *In situ* image of a fractured coral *Goniastrea retiformis* skeleton showing endolithic *Ostreobium* (green) and other phototrophs (pink). (B) Green band of *Ostreobium* as seen in a cross-section of the skeleton of the coral *Paragoniastrea australensis*. (C) *Ostreobium* bloom developing at the base of bleached *Acropora aspera* corals. (D) Free-living *Ostreobium* strains in culture dishes. (E) *Ostreobium* filaments emerging from a coral fragment in a culture dish. All images are in the public domain (Fordyce 2021, Pasella 2021, Ricci 2021, Verbruggen 2021), for details see the data availability statement below.

2. Biodiversity and evolution

Ostreobium belongs to the order Bryopsidales, which is most commonly classified in the Ulvophyceae, a class within the phylum Chlorophyta, the green algae (Fig. 2A, B). However, some uncertainty remains about the phylogenetic position of the Bryopsidales in the green algal lineage. Earlier phylogenetic analyses based on relatively small datasets suggested a sister relationship with the Dasycladales, another order of siphonous algae [17,18]. More extensively sampled chloroplast genome datasets failed to resolve clear relationships [19]. Some recent analyses based on large datasets of conserved nuclear genes recovered Bryopsidales as the sister lineage to Chlorophyceae, separated from the remaining Ulvophyceae orders (Fig. 2B, [20–22]), while other analyses retained the Bryopsidales as a sister lineage to the remaining Ulvophyceae [20]. In summary, while the Bryopsidales order is clearly an early-branching lineage of the core Chlorophyta, its exact phylogenetic placement remains elusive. As more whole-genome data accumulates for Bryopsidales and other Ulvophyceae lineages, the alternative phylogenetic hypotheses can be tested in more detail.

The *Ostreobium* clade is one of the three main lineages of Bryopsidales and has been assigned to its own suborder Ostreobineae (Fig. 2B, [23,24]). Molecular clock analyses date the divergence of the Ostreobineae from other Bryopsidales to the early Paleozoic [25], consistent with the continuous presence of trace fossils (boring tunnels) that are considered to be created by *Ostreobium* from the Ordovician onwards ([26], as *Ichnoreticulina elegans*).

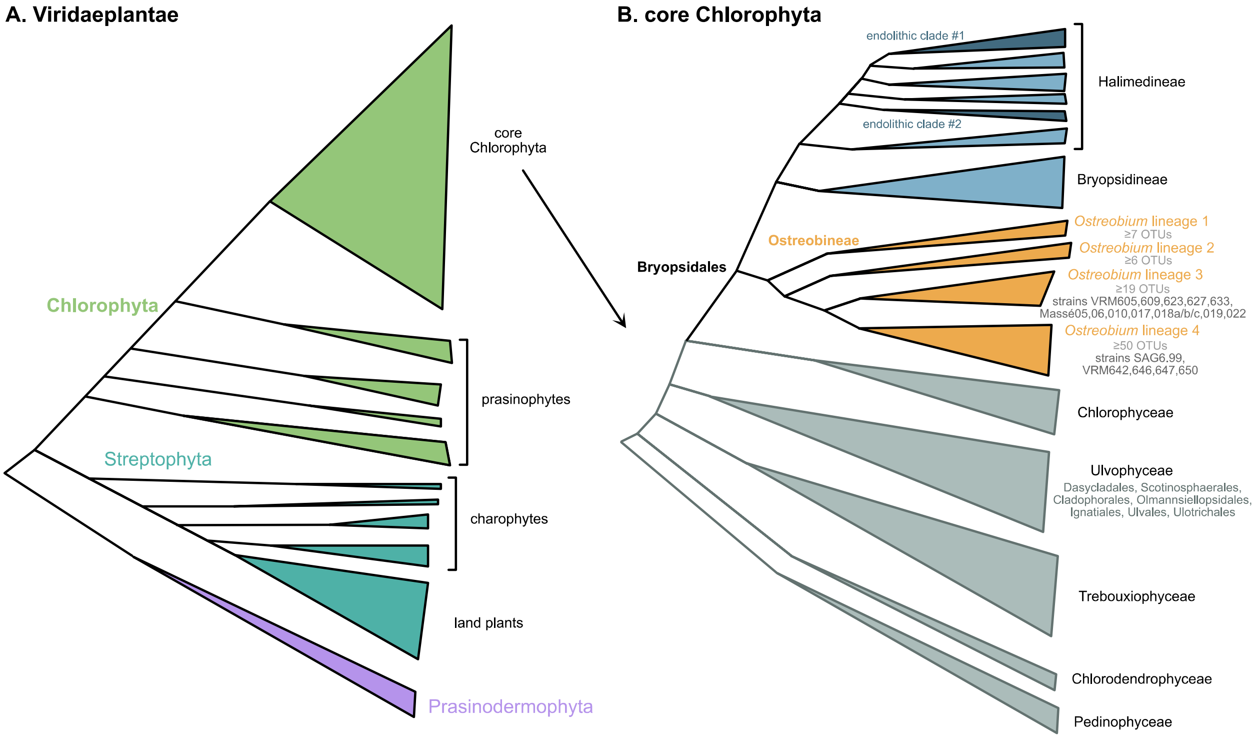
Five *Ostreobium* species are formally recognised: *O. constrictum, O. duerdenii*, *O. okamurae*, *O. reineckii* and the type species *O. quekettii*. A sixth named species (*O. brabantium*) is regarded as being rhizoids of *Acetabularia*, an unrelated genus of green algae [27]. *Ostreobium quekettii* was first described living in commercial oyster shells [28] and is widespread in various temperate and (sub)tropical carbonate substrates [29,30] over a broad bathymetric range [31,32], while *O. constrictum* has been reported only from corals and calcified red algae [29]. The other species are seldom reported, and their ecology remains unknown. For the last several decades, if a species name has been assigned to a record of *Ostreobium*, that name has almost always been *O. quekettii*.

Recent work has shown that *Ostreobium* biodiversity is underestimated by at least an order of magnitude, with environmental sequencing recovering more than 80 species-level operational taxonomic units (OTUs, [33–37]), and culture-based studies increasingly supportive of that result ([8,36], O'Kelly et al. unpublished results). The most comprehensive surveys have used the *tuf*A gene (coding for elongation factor Tu) as a DNA metabarcode [35,36] demonstrating the existence of four lineages within the Ostreobineae (Fig. 2B, [35]). The molecular divergence between these lineages is large, dating back to the early Paleozoic [35].

Environmental sequencing studies also reported two additional lineages of endolithic siphonous green algae, which evolved independently from the Ostreobineae in the bryopsidalean suborder Halimedineae (Fig. 2B). We do not know much about these lineages other than that they share the same habitat and are likely similar to *Ostreobium* in overall appearance, so it is quite likely that these would have been identified as *Ostreobium* in previous studies.

Because a large fraction of *Ostreobium* OTUs have only been recorded from metabarcoding studies, we possess only fragmentary knowledge about how species differ in their cellular, reproductive or ecophysiological traits. Cultured strains do show some differences in morphology ([9], O'Kelly et al. unpublished results), and *O. constrictum* was distinguished from *O. quekettii* based on the presence of constrictions along the siphons and chloroplast morphology [29]. However, considering the very high actual species diversity and the overall simplicity of *Ostreobium*'s structure, it seems extremely unlikely that morphological traits on their own will allow unambiguous differentiation between OTUs.

Some photophysiological differences between cultured strains were observed [38], and different genotypes of *Ostreobium* were found in coral skeletons collected at different depths [33] and from different substrates (O'Kelly et al. unpublished results), providing the first indication for ecophysiological differentiation between lineages. More recently, a meta-transcriptomic study showed that different *Ostreobium* genotypes respond differently to coral bleaching [39], and some variation in fatty acid content and isotope ratios were shown between cultured strains in free-living and limestone-burrowing states [8]. All these observations together show strong evidence that the tremendous biodiversity of *Ostreobium* is mirrored in an ecophysiological diversity that remains largely unexplored.


A. Viridaeplantae

B. core Chlorophyta

Fig. 2. Schematic representation of the phylogenetic position of *Ostreobium*. The Viridaeplantae or green lineage (A) contains three phyla including the Streptophyta that gave rise to land plants and the Chlorophyta that contain a range of green algae including the prasinophyte lineages and the core Chlorophyta. The *Ostreobium* clade (Ostreobineae) is found within the core Chlorophyta lineage (B) as

one of the three suborders within the Bryopsidales order. The four family-level lineages in *Ostreobium* are indicated along with their biodiversity and available culture strains.

3. Resources for *Ostreobium* biology

3.1 Culture strains

Only two strains have been readily available for research on *Ostreobium*. Strain SAG 6.99 was first isolated more than three decades ago as a contaminant of a red algal culture from the Philippines [40] and strain SAG 7.99 was isolated from a *Jania* species from southern Australia. Both these strains were identified as *O. quekettii* but molecular data show they cluster in different lineages [33]. Another strain (B14.86) was used to study morphology, reproduction, photoecology and trophic potential [11,41] but is not currently available from any culture collection.

With the renewed interest in *Ostreobium*'s roles in coral reef ecosystems, there has been a surge in new strain cultivation, and 21 strains are now available from public repositories (Table 1). The majority of these strains were isolated from coral skeletons, including nine from *Pocillopora acuta* at the Aquarium Tropical du Palais de la Porte Dorée (Paris, France; originally from Indonesia) deposited at the RBCell collection (Biological Resources of Living and Cryopreserved Cells; Paris, France) [8]. The remaining ten strains were isolated from the skeletons of Great Barrier Reef corals, eight from *Porites* sp*.,* one from *Pavona* sp*.* and one from *Merulina* sp*.* [9], and these strains are available from the Australian National Algal Culture Collection (ANACC, Hobart). Additional strains are in the personal collections of author O'Kelly (deposition in public collections pending) and of Thomas Sauvage (pers. comm.).

3.2 Genomic resources

A range of genomic resources has been generated for *Ostreobium* in the last few years including the chloroplast, mitochondrial and nuclear genomes.

Complete chloroplast genomes have now been published for 14 *Ostreobium* strains. All are similar in size (~80kb), GC-poor (32%), gene dense and encode a set of core genes for photosynthesis, transcription, translation and ATP generation very similar to the gene set found in other Bryopsidales, and they feature only a handful of introns [23,42,43]. While *Ostreobium* chloroplast genomes are smaller than those of most other green algae, the opposite is true of their mitochondrial genomes, with the *O. quekettii* mitochondrial genome (242kb) being the largest currently known for green algae [42,44]. The expanded mitochondrial genome of *Ostreobium* is rich in intergenic DNA, shows a proliferation of group II introns and has a less biased base composition (48% GC) than the chloroplast [44]. The occurrence of large mitochondrial genomes alongside small chloroplast genomes appears to be a characteristic of the Bryopsidales that deviates from other Chlorophyta, where mitochondrial genomes are typically small and intron-poor [44–46]. The small size of the chloroplast genome was initially attributed in part to resource limitation due to its low-light habitat [42], but the newer knowledge on the mitochondrial genome shows that the two organelle genomes in *Ostreobium* have experienced different evolutionary processes [44].

The draft nuclear genome assembly of SAG6.99 is 146Mb and codes for 10,633 protein-coding genes. Although this genome is still fragmented (2,857 contigs, [39]), it allowed for comparison with published nuclear genomes from other green algae including the Ulvophyceae *Ulva* *mutabilis* [47] and *Caulerpa lentillifera* [48]. The overall genome structure of *Ostreobium* is similar to other members of the green algal lineage, with the percentage of genes with introns (81.6%) being similar to the other Ulvophyceae (*C. lentillifera* 80% and *U. mutabilis* 85%), but the GC content (52.4%) reportedly higher compared to *C. lentillifera* (40.4%), and lower than *U. mutabilis* (57.2%) [39]. The assembled genome revealed an expansion of genes related to calcium transport, oxidative stress response and photosynthesis, reflecting its adaptation to an endolithic niche [39].

4. *Ostreobium* as a laboratory organism

4.1 Morphological structure

The basic structural unit of a living *Ostreobium* is the coenocytic (siphonous) filament, as is the case for all members of the Bryopsidales [7]. These filaments are long, branched tubes of cytoplasm without cross walls. *Ostreobium* differs from most other Bryopsidales in that the filaments are not organized into a macroscopic, three-dimensional algal body. *Ostreobium* strains also have much narrower siphons than other Bryopsidales, with vegetative filaments of an *Ostreobium* typically less than 10 µm in diameter when grown in free culture and less than 5 µm in diameter when in $CaCO_3$.

The filaments contain a large number of small, discoidal plastids (Fig. 3A) and are multinucleate (Fig. 3B). All plastids are chloroplasts (i.e., no amyloplasts) and lack pyrenoids. Because of the small diameter of the filaments, the chloroplasts are usually closely packed, and their shape and size can be difficult to discern. Filament elongation occurs exclusively at filament apices, and they may expand in width, as can be seen in swellings, and, to a lesser degree, indeterminate laterals and older axes.

Filaments are branched, with the branching pattern depending on the alga's habitat and growth conditions. The most complex branching patterns are seen when the alga is growing in $CaCO_3$, and its filaments can reach the surface of the mineral. The alga's gross structure then consists of a single central axis, from which indeterminate laterals branch off at intervals (Fig. 3C). Subsequent branching of these laterals, at third or higher orders, results in the formation of determinate laterals that form interlocking net-like patterns at the surface of the substrate (Fig. 3D, E). The observed pattern is diagnostic for *Ostreobium* (and its trace fossil counterpart *Ichnoreticulina*). This two-dimensional pattern is similar to those found in many macroscopic Bryopsidales, with their central axial filaments and determinate laterals (utricles) arranged in three dimensions.

Branching patterns are much reduced when *Ostreobium* is reared in free culture (liquid or agar-solidified, no solid $CaCO_3$), and when filaments growing in $CaCO_3$ do not reach the mineral's surface. Under these conditions, determinate laterals are rare or absent, and only indeterminate axes and laterals are present (Fig. 3F).

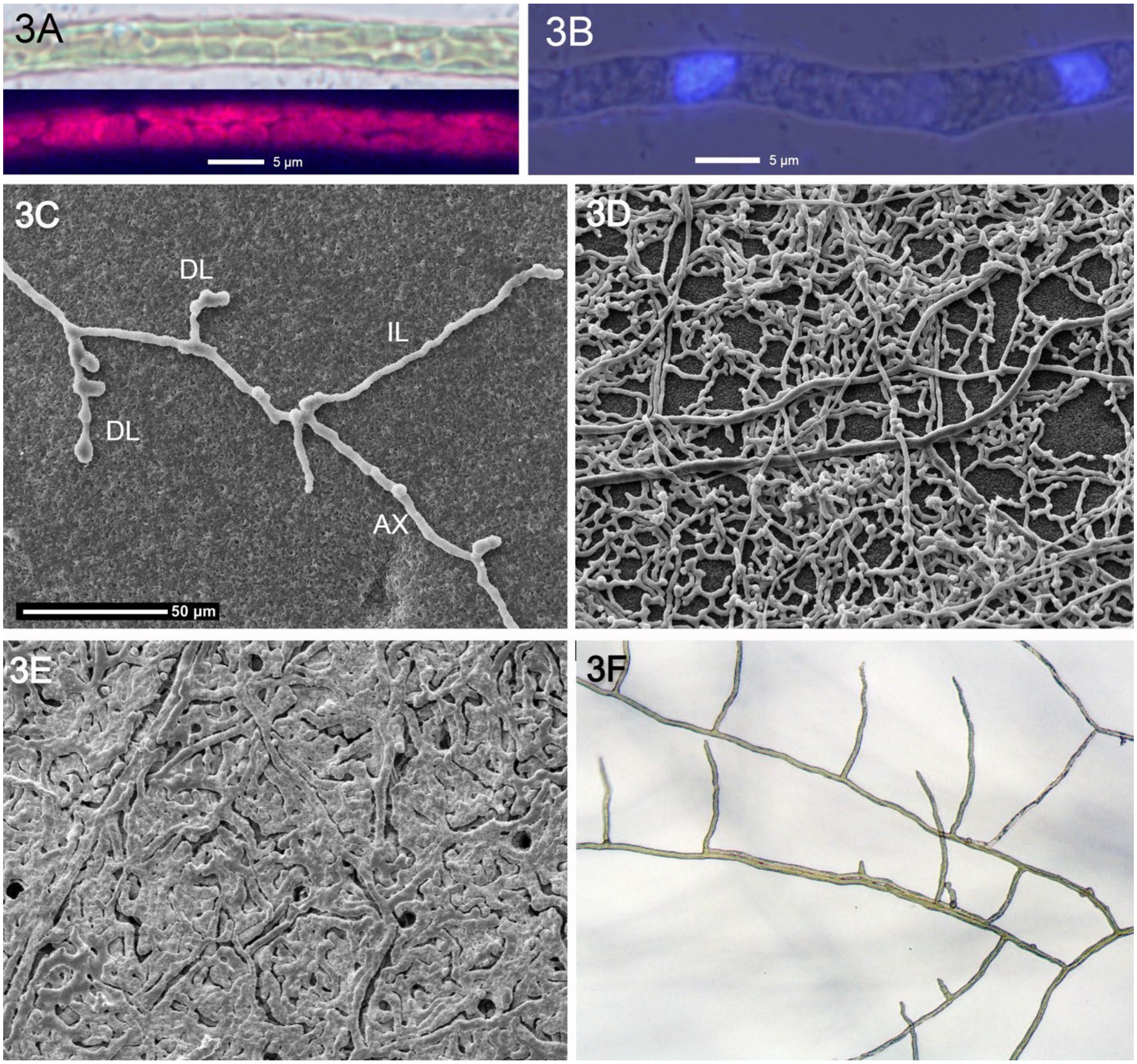


Fig. 3: Details of *Ostreobium* structure. (A) Segment of living filament with close-packed small discoidal chloroplasts, in bright field (top) and fluorescence microscopy. (B) Segment of DAPI-stained filament with two nuclei. Composite bright-field and fluorescence image. (C) SEM of a resin cast showing filament from the margin of a boring, invading previously unoccupied matrix, showing main axis (AX), an indeterminate lateral (IL), and two determinate laterals (DL). (D) SEM of a resin cast showing filaments from near the center of a 1-month-old boring. Determinate laterals have not completely filled the space left by axes and indeterminate laterals. (E) SEM of a resin cast showing filaments from near the center of a 3-month-old boring, with determinate laterals largely filling the space. Axes and indeterminate laterals (branches) can still be discerned. (F) Filaments of a possibly axenic culture growing in agar. Determinate laterals are not present. Scale bar in C also applies to D-F. Images are available in the public domain, for details see the data availability section below.

4.2 Isolation and culturing method

As is the case for all endolithic algae, isolation of live *Ostreobium* from carbonate substrates is challenging. The coenocytic nature of *Ostreobium* filaments magnifies the challenge, as does the extreme rarity of sporulation. While some cells of other species will survive mechanical maceration of the substrate, and these cells can then be selected and grown, *Ostreobium* coenocytes will not. Ethylenediaminetetraacetic acid (EDTA) has been used to dissolve the carbonate, leaving live algal cells to be extracted and reared in culture [49,50]. However, the method is slow and works only on small pieces of substrate.

The most successful way to isolate *Ostreobium* has been to place substrate fragments bearing the target alga into a liquid culture medium and wait for the filaments of the alga to grow outside the substrate (Fig 1E; [8,9], O'Kelly et al. unpublished results), after which they may be isolated using standard manual methods for cell isolation and growth [51]. Excision of clean millimeter-scale fragments of substrate bearing *Ostreobium*, which are then used as starting material, can increase the chances of success.

No special media are required; *Ostreobium* will grow in any of the standard seawater-enrichment media [52]. However, very low visible light levels (400-700 nm), on the order of 5 µmol photons m^{-2} s^{-1}, typically have been required for successful isolations. Higher irradiance levels risk killing the *Ostreobium* while promoting contaminants. The low-light requirement seems to be common to all low-light-adapted endolithic algae and cyanobacteria. The isolation process is typically slow and labor-intensive, requiring frequent monitoring of the cultures over several weeks to observe the target alga and remove filaments for unialgal cultivation at the earliest possible moment, while contaminants are yet manageable. Helpful tools are flat-bottomed well plates, with the wells large enough to allow manipulation of tiny algal pieces (e.g., 12- and 24-well plates) and an inverted microscope for monitoring. Judicious use of antibiotics and germanium dioxide can help keep cyanobacteria and diatoms at bay.

Unialgal cultures of *Ostreobium* can be reared either as free-living filaments, in liquid or agar-solidified media, or in a carbonate substrate ([8,9], O'Kelly et al. unpublished results). Actively growing filaments in liquid culture are typically competent to infiltrate solid carbonates, which happens exclusively by penetration of the substrate by filament apices.

Ostreobium filaments penetrate agar, and this is probably the most suitable way to produce axenic strains, as filament apices that have grown away from contaminants can be excised and inoculated into fresh medium. Antibiotic applications can help control contaminating bacteria, but if the initial unialgal culture does not contain agar-penetrating microbes, it may be possible to establish axenic cultures without using them. No information has yet been published on the bacteria associated with *Ostreobium* strains, and no confirmed axenic strains of *Ostreobium* currently exist, but this work is ongoing. Other genera of Bryopsidales show tight associations with bacteria, including symbiotic bacteria living inside the siphonous filaments [53,54], so obtaining completely bacteria-free strains may not be possible or practical.

Protoplast formation is another potential culturing tool that has been developed for other Bryopsidales but has hardly been used in *Ostreobium*. *Bryopsis plumosa* (Huds.) Ag. has been shown to generate about a thousand new cells from a single mature filament following protoplast formation [55]. Traditionally, protoplast formation has been associated with wound healing [56,57], but the generation of viable new cells from a wounded cell in *B. plumosa* highlights its potential as a method of propagation [55]. Developing protoplast formation methods in *Ostreobium* would aid culturing efforts and have a range of other applications in developing this alga as a model system, with the potential to increase our understanding of cell organelle interaction and provide insights into cell membrane formation and the evolution of siphonous structure in green algae.

4.3 Propagation and life history

The principal mode of propagation known for *Ostreobium* is vegetative. Laboratory cultures can be propagated by fragmentation of the culture mass, despite the coenocytic nature of the filaments. Presumably, broken *Ostreobium* filaments undergo a wound-healing response like in the related genus *Bryopsis*, mediated by the lectin bryohealin [58].

Ostreobium can disperse via released zoospores [9,41]. The long exit tubes observed on *Ostreobium* zoosporangia/swellings [41] are reminiscent of those found on other endolithic chlorophytes such as *Phaeophila dendroides* [59] and the codiolum-stage sporophytes of the *Gomontia* group of Ulotrichales [60], where they serve to reach the carbonate surface and expel propagules (zoospores) into the surrounding medium.

Sexual reproduction has not been observed in *Ostreobium*, and there are few clues available to assess whether it exists, and, if it does, what form it takes. It is intriguing to see that the

Ostreobium draft genome lacks several of the genes considered essential for meiosis [18]. These missing genes are mostly involved in recombination and chromosomal pairing during meiosis, e.g., DNA meiotic recombinase 1 (DMC1) and homologous pairing protein 1 and 2 (HOP1 and HOP2) [18,61]. Although theoretically essential, HOP1 and HOP2 are also absent in *Caulerpa lentillifera*, another Bryopsidales for which the genome is available. Yet despite these absent genes, sexual reproduction has been observed in several Bryopsidales [62] including *Caulerpa*. Missing some meiosis-related genes does not imply asexuality [18,63], and given how immature our knowledge on the genomic basis of reproduction in Bryopsidales is, inferring asexuality of *Ostreobium* only from these genomic clues seems premature.

It has been reported that the quadriflagellate zoospores released by *Ostreobium quekettii* culture strain produce filaments identical in size and structure to the parent [41]. The progeny remained vegetative, so it is unknown whether the zoosporic reproduction was part of a sexual cycle. Quadriflagellate zoospores are commonly observed among Ulvophyceae and some groups of Chlorophyceae, but no other member of Bryopsidales is known to have them. In these other taxa, quadriflagellate zoospores may be produced by mitosis, and replicate the parent plant, or by meiosis and yield the gamete-producing generation (gametophyte) in an alternation of (typically) diploid and haploid life history phases. Several Bryopsidales, especially species of the Bryopsidineae, have life histories consisting of an alternation of heteromorphic phases. In some of these species, including *Bryopsidella ostreobiformis* [64], the meiospore-producing generation (sporophyte) may have a creeping base somewhat reminiscent of *Ostreobium*. A detailed exploration of these hints has yet to be undertaken.

4.4 Growth rates

Cultures of *Ostreobium* grow slowly under all conditions tested to date. For example, it typically takes 1-2 months from the initiation of a culture into a 1 cm^2 carbonate substrate to complete coverage of that substrate. Standard methods used to measure growth in other algae do not transfer well. For example, chlorophyll measurements may not provide accurate indications of growth, as *Ostreobium* in liquid culture typically grows in a three-dimensional mass (Fig. 1), and self-shading of the interior of the mass can affect the measurements. Moreover, only a few experimental studies have explored links between light availability, photosynthesis and growth of *Ostreobium*, and because of the long evolutionary history of this genus and the considerable genetic diversity among strains, divergent mechanisms may also be present. Temperature effects on growth are also poorly known. Most strains from cold-temperate environments grew in the culture at both 15ºC and 23ºC, but strains from tropical environments died at 15ºC (O'Kelly et al. unpublished results).

5. A model for low light photosynthesis

The natural habitats in which *Ostreobium* is found clearly illustrate its affinity for low light. *Ostreobium* lives as an endolithic alga in solid carbonate substrates, severely limiting the light it receives. The genus has also been found at water depths of over 200 m, under light exposure of less than 0.001% of the surface irradiance [2,65]. Within the coral skeleton, *Ostreobium* lives in the shade of the Symbiodiniaceae, with the photosynthetically active radiation (PAR) reaching the *Ostreobium* band ranging between 0.01% and 2% of the irradiance at the coral surface [11,66–68].

Its extremely energy-limited environment has led to a few studies on *Ostreobium* photosynthesis. Experiments on culture strains showed saturation of photosynthesis between 35 to 200 µmol photons $m^{-2}s^{-1}$ [11,69], but a study using optical sensors for *in situ* measurements of photosynthesis in the green *Ostreobium* layer in coral skeleton showed that this alga was saturated at even lower irradiance of ~7 µmol photons $m^{-2}s^{-1}$ [70].

Ostreobium also has the ability to perform oxygenic photosynthesis in a spectral range that most other algae do not use, i.e., the far-red spectral region comprising particularly near-infrared radiation (NIR) of ~700-750 nm [39,71]. NIR-driven photosynthesis is primarily relevant in shallow water habitats since NIR wavelengths are readily absorbed by water and unavailable beyond a few meters' depth. It is a particularly beneficial trait in habitats shaded by other phototrophs such as those where *Ostreobium* thrives, in the shade of Symbiodiniaceae in the coral skeleton, or under a canopy of seaweeds and/or crustose calcareous algae in the reef matrix.

The ability for NIR-driven oxygenic photosynthesis is not exclusive to *Ostreobium*. In cyanobacteria, many of which live in similar habitats to *Ostreobium*, specialised chlorophylls (chl *f* and chl *d*) absorb further in the far-red spectrum but these pigments are not found in *Ostreobium* [72–75]. There is a need for more detailed studies of the interactions between the photosystems (PS) and antenna pigments (e.g., the light-harvesting complex - LHC) in *Ostreobium* to explain its ability to use NIR light. An uphill energy transfer was hypothesised in *Ostreobium* [71], as has been already reported in higher plants, green algae and *cyanobacteria* [76–78], however, the mechanism in *Ostreobium* appears to involve an atypical association between PSII and the Lhc1a containing long-wavelength chlorophylls.

The *Ostreobium* nuclear genome presents a rich repertoire of light-harvesting complex proteins in both photosystems [39]. Most green algae possess only one copy of the *Lhca1* gene, an important light-harvesting complex protein in photosystem I, but this gene is duplicated in *Ostreobium*. Additionally, *Ostreobium Lhca1* genes present a mutation in a chlorophyll-binding residue that suggests it may absorb far-red light [39,79,80]. *Ostreobium* also has an unusual composition of the light-harvesting complex protein family, including an unusual combination of *Lhcb* and *Lhcp*. Most green algae have *Lhcb* but the prasinophytes have *Lhcp* instead [81]. How these genes are being used in *Ostreobium* has not yet been determined. The adaptation to low light is also reflected in the loss of many genes for photoprotection, including non-photochemical quenching (NPQ) genes and PsbS and photoreceptors from the *Ostreobium* genome [39].

The knowledge that *Ostreobium* is a slow-growing, low light-adapted species is difficult to reconcile with the observation that when much higher light levels reach into the coral skeleton during coral bleaching, blooms of *Ostreobium* can occur. *In situ* studies have suggested that the increase of both *Ostreobium* biomass and chlorophyll is due to efficient light acclimation [13,82]. Ongoing work indicates that NPQ, a common photoprotective mechanism employed by algae for high light acclimation, does not appear a predominant mechanism in *Ostreobium* (Pasella et al., unpublished results). Which mechanisms are employed by *Ostreobium* to deal with high irradiance remain unknown, but ongoing work indicates a high repair rate of the D1 protein responsible for binding the primary electrons donors and acceptors in the PSII. However, as described for other algae [83], an elevated D1 repair rate is not a realistic long-term mechanism for high-light adaptation. The xanthophyll cycle is another common mechanism to dissipate excess energy, but both pigment profiles and genome information show that this cycle does not exist in *Ostreobium* and probably more broadly in Bryopsidales [39,84,85].

In summary, it is clear that *Ostreobium* is a good model to improve our understanding of green algal photosynthesis in unusual light environments, but how it achieves efficient light-harvesting and avoids damage from excess light exposure during coral bleaching remains unknown. Furthermore, it appears that the photosynthetic traits of *Ostreobium* strains differ from each other ([38], Pasella et al. unpublished results), adding to the knowledge gaps in *Ostreobium* photobiology.

6. A model for endolithic growth

Endoliths like *Ostreobium* can actively excavate microscopic galleries in the carbonate substrates they colonize, including coastal and terrestrial limestones. *Ostreobium* is estimated to dissolve >1kg of $CaCO_3$ per m^2 of tropical coral reef per year [6] and is regarded as one of the most potent microbial bioeroders in tropical environments [4,86]. In marine environments, which are supersaturated for calcite and aragonite, bioerosion via dissolution of carbonate is thermodynamically unfavorable, especially in microenvironments where carbon fixation by oxygenic phototrophs increases the pH of the surrounding microenvironment. This increase in pH facilitates precipitation of carbonate instead of dissolution, rendering the boring process by oxygenic phototrophs paradoxical [87,88]. Endoliths have mechanisms that overcome this challenge (see below), but these are yet to be understood comprehensively. Gaining an understanding of these mechanisms will help evaluate how ocean acidification is likely to affect reef bioerosion.

At present, much of our physiological understanding of life as an endolith stems from studies on the cultured endolithic cyanobacterium *Mastigocoleus testarum*. A mechanism centered on three plausible hypotheses of limestone boring by microbial phototrophs: (1) temporal separation between photosynthesis and boring, with the former occurring during the day and the latter during the night, (2) spatial separation between photosynthesis and boring along the apical axis, and (3) transport of Ca^{2+} from extracellular spaces at the site of excavation into the cell and internal translocation away from the site of excavation, has been proposed [88]. These hypotheses were evaluated in *M. testarum* BC008, showing that this cyanobacterium uses transcellular Ca^{2+} transport during boring with protons constituting the counter ions exchanged for Ca^{2+} to maintain the charge through P-type ATPases [89]. Specific adaptations in BC008 for long-range active Ca^{2+} pumping by multiple cells along the boring front, orchestrated by polarized localization of calcium pumps in a ring pattern at one end of the cell was recently reported [90]. BC008 was also shown to harbor specialized cells called calcicytes, which can accumulate 500-fold higher concentrations of calcium as compared to other cyanobacteria [90]. These calcicytes likely can act as storage buffers for excess calcium and allow the flow of calcium at nontoxic concentrations through non-calcicyte cells before excretion to the surrounding environment [90].

These findings shed light on the boring mechanism and related adaptations, but the question remains how universal this mechanism is beyond *Mastigocoleus*. The mechanism of carbonate excavation in *Ostreobium* has not yet been characterised, but a general model, based on the *Mastigocoleus* template and gleaning insights from the *Ostreobium* nuclear genome, which is very rich in calcium transport-related genes, was recently proposed [39]. This model requires validation, and the roles of specific transporters are yet to be determined. It seems likely that *Ostreobium*, similar to cyanobacteria, uses a temporal separation between photosynthesis and burrowing, as recent studies showed that a higher rate of $CaCO_3$ dissolution was observed at night [91]. The siphonous nature of *Ostreobium* might also aid in the spatial separation of photosynthesis from the boring front by intracellular translocation of chloroplasts as observed in other Bryopsidales [92], but this has not been studied yet.

Unlike most algae that live in oxygen-rich environments, *Ostreobium,* in general, does not. In the skeletons of most corals, aerobic conditions are only found in those areas colonized by *Ostreobium* and probably the skeleton of most corals experiences no more than about 60% oxygen saturation as reported for *Porites* sp. [73]. Any oxygen produced by photosynthesis in the coral skeleton is depleted in a matter of minutes after darkness, making the environment anoxic for long periods, although a few pockets of higher oxygen may be maintained for longer [69]. While we do not have a complete picture of all the adaptations of *Ostreobium* to this environment, its genome does suggest it has a range of fermentation options and shows an expansion of lactate, aldehyde and malate dehydrogenases [39].

7. *Ostreobium* as a member in the coral holobiont

Much of the recent attention on *Ostreobium,* including the sequencing of its nuclear genome, has focused on deciphering its role in the coral holobiont. The onset of *Ostreobium* colonization of its coral host has been shown in very young (7-day old) coral recruits, when calcification begins [37]. However, for this colonization to happen, *Ostreobium* strains have to be present in the settlement substrate, and *Ostreobium* biodiversity likely changes during the host's life. In adult corals, typically only one or two *Ostreobium* genotypes are dominant, whereas *Ostreobium* diversity in young recruits is largely dependent on the composition of the rock on which coral planula larvae settle [37]. The abundance and diversity of *Ostreobium* genotypes also vary across coral species, with massive corals harboring more diversity than branched corals [93,94].

Ostreobium's role in the coral holobiont remains far from being resolved and a few questions need urgent attention, including (i) whether and how *Ostreobium* supports the coral host and other members of the holobiont, (ii) how the beneficial and detrimental effects of *Ostreobium* balance out across the coral's life span, and (iii) how *Ostreobium* depends on the coral host and other holobiont members. These questions have become important under the evident and increasing threats of global warming to coral reefs. In a healthy coral, endosymbiotic dinoflagellates (Symbiodiniaceae) provide the bulk of the coral's carbohydrate needs, but this relationship is interrupted by prolonged exposure to high temperatures, leading to the loss of Symbiodiniaceae (coral bleaching) and coral death if the bleaching is not reversed.

In bleached corals that are almost devoid of Symbiodiniaceae, more light penetrates the skeleton, leading to *Ostreobium* blooms due to their increased photosynthetic activity [95], and photosynthates generated by *Ostreobium* may become an important source of carbohydrate for the coral host. *Ostreobium* provides photosynthates to the host during bleaching. ^{14}C labelled bicarbonate was used to show that ~27% of the total ^{14}C-photoassimilates were incorporated into the tissue lipids of the azooxanthellate coral *Tubastrea micranthus* [11]. Similar experiments on zooxanthellae-harboring corals have all pointed to a role of *Ostreobium* in nutrient recycling and transfer of photoassimilates from endoliths to the coral tissue [11–13,82]. Together, these studies indicate that *Ostreobium* might act as a crucial temporary lifesaver for corals during bleaching. However, the circumstances in which *Ostreobium* photosynthesis can sustain coral life and any secondary effects that the increased skeletal photosynthate production may have on the coral via other holobiont members remain to be studied.

The *Ostreobium*-coral symbiotic relationship also has benefits for the algae, including shelter in the skeleton and potentially the transfer of CO_2 and nitrogen-containing metabolites from the host, although this has not been demonstrated [96]. Analysis of the *Ostreobium* genome and coral skeleton meta-transcriptomes indicate that *Ostreobium* depends on other holobiont partners, most likely bacteria, for vitamin B12 [39]. Endolithic fungi have been shown to penetrate filaments of *Ostreobium* spp., but the nature of these interactions remains to be resolved [97].

Many aspects of *Ostreobium*'s role in the coral holobiont are not fully understood. While we know that 7-day old coral nubbins are colonized by *Ostreobium* filaments [37], what sort of signaling (if any) mediates or triggers this colonization remains to be studied, as are the effects of early *Ostreobium* colonisation on coral recruits. Astonishingly, *Ostreobium* has been retrieved from mesophotic corals down to the limits of the photic zone in tropical waters [2,3,98]. This begs the question of whether and how a low-light specialist like *Ostreobium* could contribute to coral survival at those depths and whether this interaction benefits both partners. Even for shallow water corals, the relative contribution of *Ostreobium* and Symbiodiniaceae to the coral's carbon budget has not been quantified comprehensively. We do not yet have a detailed picture of the metabolic interactions between coral and *Ostreobium* or of the overall balance of positive and negative effects of *Ostreobium* on the coral across its lifecycle. How physiological diversity between *Ostreobium* species might affect these interactions also remains largely uncharted terrain.

8. Conclusion

Despite its interesting biology, *Ostreobium* is not an established model organism. The recent publication of its nuclear genome is a significant step forward. Classical genetic tools like outcrossing are not currently available and given that the life cycle is yet to be determined, such tools are still a way off. Nonetheless, progress could be made using forward genetic screens on spores or by developing a protoplast formation protocol like that available for *Bryopsis* [55].

Similarly, transformation, gene editing and RNAi procedures are yet to be developed. Transformation attempts in *Bryopsis* have only been able to achieve transient transformation (Gwang Hoon Kim, pers. comm.), possibly due to the multinucleate nature of these siphonous cells. The diploid nature of the *Ostreobium* SAG6.99 strain, for which the genome has been sequenced, may further complicate transformation. Gaining control over sporulation or protoplast formation may thus be key to developing transformation protocols.

The slow growth and lack of knowledge about *Ostreobium*'s sexual cycle limit its use for some applications, and it may be beneficial to have a second model organism in Bryopsidales that can be transformed with *Ostreobium* genes to test hypotheses. *Bryopsis* appears to be a good candidate, with a known life cycle, rapid growth, mass protoplast formation, and a growing understanding of its ultrastructure, developmental and molecular biology. Though the *Bryopsis* genome sequencing is in progress (Gwang Hoon Kim, pers. comm.), additional efforts are necessary to develop transformation protocols and genetic tools for this organism.

Even without an extensive genetic toolkit, there is much that can be learned about the basic biology of *Ostreobium*. The nuclear genome has provided hints at *Ostreobium* photobiology and bioerosion, but experimental work will be needed to test specific hypotheses about the underlying physiological mechanisms. The boring mechanism of *Ostreobium*, which likely includes carbonate dissolution at the growth tip and the potential use of cytoplasmic domains or vacuoles for Ca^{2+} storage and/or transit as suggested [39], But to validate genome-based hypothesis and improve our understanding of *Ostreobium* bioerosion, will require microscopic studies of Ca^{2+} translocation combined with localisation of the putative transporters involved.

Recent studies have shed light on *Ostreobium's* role in providing photosynthates to coral hosts during bleaching, but almost nothing is known about the array of metabolic interactions that potentially take place between the coral, *Ostreobium* and other microbiome members. The addition of new coral genomes and the availability of the *Ostreobium* genome can now facilitate a range of experimental work, including microbiome, transcriptomic and metabolomic studies to understand the coral-*Ostreobium* interaction.

Ostreobium photobiology can now proceed with an abundance of strains available for *in vitro* experiments. Ongoing work focuses on how *Ostreobium* acclimates and adapts to different niches and performs photosynthesis, while the availability of the genome offers perspectives to delve deeper into the molecular mechanisms as well. The genome already has provided some hints about *Ostreobium*'s adaptation to extremely low light environments and its ability for NIR-driven photosynthesis [39], but more work will have to be done to investigate how it uses its expanded light-harvesting protein repertoire.

Last but not least, *Ostreobium* taxonomy needs a thorough overhaul. Systematic interpretation of the genetic diversity within the genus is hampered by a lack of information about the type species, *O. quekettii*. Consequently, no Linnaean nomenclature has developed, and entities within the genus are referenced by strain, sample, or DNA barcode designators. The distribution of individual genotypes in natural habitats and the spreading mechanisms for different genotypes remains poorly understood. Extensive sampling, culturing, and molecular biology analyses at a series of defined locations will be needed to better grasp *Ostreobium* population biology. As a necessary foundation for taxonomic work, *O. quekettii* will need to be (re)defined, from specimens collected at Le Croisic, France where *Ostreobium* was first discovered and described.

We are still in the very early stages of discovering the biology of *Ostreobium* and hope that the ideas and resources outlined in this paper will encourage the scientific community to contribute to advancing the knowledge about this highly unusual alga.

Data Availability

All figures presented in this review are available for re-use under license (CC BY 4.0) at https://figshare.com/articles/figure/Ostreobium_as_model_system/16786507. We have built a web page with information on *Ostreobium* resources, including publicly available strains and genome data: https://hverbruggen.github.io/resources/Ostreobium_Resources.html. This builds upon Table 1 of this paper but includes further links to genome data and we aim to keep this updated as more data become available.

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Declaration of interest

None

References

[1] H.T. Odum, E.P. Odum, Trophic structure and productivity of a windward coral reef community on Eniwetok atoll, Ecol. Monogr. 25 (1955) 291–320.

[2] M.M. Littler, D.S. Littler, S.M. Blair, J.N. Norris, Deepest known plant life discovered on an uncharted seamount, Science. 227 (1985) 57–59.

[3] K. Vogel, M. Gektidis, S. Golubic, W.E. Kiene, G. Radtke, Experimental studies on microbial bioerosion at Lee Stocking Island, Bahamas and One Tree Island, Great Barrier Reef, Australia: implications for paleoecological reconstructions, Lethaia. 33 (2000) 190–204.

[4] A. Tribollet, The boring microflora in modern coral reef ecosystems: a review of its roles, Current Developments in Bioerosion. (2008). https://link.springer.com/chapter/10.1007/978-3-540-77598-0_4.

[5] F. Ricci, A. Fordyce, W. Leggat, L.L. Blackall, T. Ainsworth, H. Verbruggen, Multiple techniques point to oxygenic phototrophs dominating the Isopora palifera skeletal microbiome, Coral Reefs. 40 (2021) 275–282.

[6] J.S. Grange, H. Rybarczyk, A. Tribollet, The three steps of the carbonate biogenic dissolution process by microborers in coral reefs (New Caledonia), Environ. Sci. Pollut. Res. 22 (2015) 13625–13637.

[7] P.S. Vroom, C.M. Smith, The challenge of siphonous green algae, Am. Sci. 89 (2001) 525–531.

[8] A. Massé, A. Tribollet, T. Meziane, M.-L. Bourguet-Kondracki, C. Yéprémian, C. Sève, N. Thiney, A. Longeon, A. Couté, I. Domart-Coulon, Functional diversity of microboring Ostreobium algae isolated from corals, Environ. Microbiol. 22 (2020) 4825–4846.

[9] M.M. Pasella, M.-F.E. Lee, V.R. Marcelino, A. Willis, H. Verbruggen, Ten Ostreobium (Ulvophyceae) strains from Great Barrier Reef corals as a resource for algal endolith biology and genomics, BioRxiv. (2021) 2021.08.16.453452. https://doi.org/10.1101/2021.08.16.453452.

[10] L.M. Ferrer, A.M. Szmant, Nutrient regeneration by the endolithic community in coral skeletons, in: Proceedings of the 6th International Coral Reef Symposium, AIMS Townsville, Australia, 1988: pp. 1–4.

[11] D. Schlichter, H. Kampmann, S. Conrady, Trophic potential and photoecology of endolithic algae living within coral skeletons, Mar. Ecol. 18 (1997) 299–317.

[12] L. Sangsawang, B.E. Casareto, H. Ohba, H.M. Vu, A. Meekaew, T. Suzuki, T. Yeemin, Y. Suzuki, 13C and 15N assimilation and organic matter translocation by the endolithic community in the massive coral Porites lutea, R Soc Open Sci. 4 (2017) 171201.

[13] M. Fine, Y. Loya, Endolithic algae: an alternative source of photoassimilates during coral bleaching, Proc. Biol. Sci. 269 (2002) 1205–1210.

[14] F. Ricci, V. Rossetto Marcelino, L.L. Blackall, M. Kühl, M. Medina, H. Verbruggen, Beneath the surface: community assembly and functions of the coral skeleton microbiome, Microbiome. 7 (2019) 159.

[15] M. Pernice, J.-B. Raina, N. Rädecker, A. Cárdenas, C. Pogoreutz, C.R. Voolstra, Down to the bone: the role of overlooked endolithic microbiomes in reef coral health, ISME J. 14 (2019) 325–334.

[16] Claudia Tatiana Galindo-Martínez, Michele Weber, Viridiana Avila-Magaña, Susana Enríquez, Hiroaki Kitano, Mónica Medina & Roberto Iglesias-Prieto, The role of the endolithic alga Ostreobium spp. during coral bleaching recovery, Sci. Rep. (n.d.). https://doi.org/10.1038/s41598-022-07017-6.

[17] E. Cocquyt, H. Verbruggen, F. Leliaert, O. De Clerck, Evolution and cytological diversification of the green seaweeds (Ulvophyceae), Mol. Biol. Evol. 27 (2010) 2052–2061.

[18] K. Fučíková, F. Leliaert, E.D. Cooper, P. Škaloud, S. D'Hondt, O. De Clerck, C.F.D. Gurgel, L.A. Lewis, P.O. Lewis, J.M. Lopez-Bautista, C.F. Delwiche, H. Verbruggen, New phylogenetic hypotheses for the core Chlorophyta based on chloroplast sequence data, Frontiers in Ecology and Evolution. 2 (2014) 63.

[19] C. Jackson, A.H. Knoll, C.X. Chan, H. Verbruggen, Plastid phylogenomics with broad taxon sampling further elucidates the distinct evolutionary origins and timing of secondary green plastids, Sci. Rep. 8 (2018) 1523.

[20] A. Del Cortona, C.J. Jackson, F. Bucchini, M. Van Bel, S. D'hondt, P. Škaloud, C.F. Delwiche, A.H. Knoll, J.A. Raven, H. Verbruggen, K. Vandepoele, O. De Clerck, F. Leliaert, Neoproterozoic origin and multiple transitions to macroscopic growth in green seaweeds, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 2551–2559.

[21] Ø.S. Gulbrandsen, I.J. Andresen, A.K. Krabberød, J. Bråte, K. Shalchian-Tabrizi, Phylogenomic analysis restructures the ulvophyceae, J. Phycol. 57 (2021) 1223–1233.

[22] X. Li, Z. Hou, C. Xu, X. Shi, L. Yang, L.A. Lewis, B. Zhong, Large Phylogenomic Data sets Reveal Deep Relationships and Trait Evolution in Chlorophyte Green Algae, Genome Biol. Evol. 13 (2021). https://doi.org/10.1093/gbe/evab101.

[23] H. Verbruggen, V.R. Marcelino, M.D. Guiry, M.C.M. Cremen, C.J. Jackson, Phylogenetic position of the coral symbiont Ostreobium (Ulvophyceae) inferred from chloroplast genome data, J. Phycol. 53 (2017) 790–803.

[24] M.C.M. Cremen, F. Leliaert, J. West, D.W. Lam, S. Shimada, J.M. Lopez-Bautista, H. Verbruggen, Reassessment of the classification of Bryopsidales (Chlorophyta) based on chloroplast phylogenomic analyses, Mol. Phylogenet. Evol. 130 (2019) 397–405.

[25] H. Verbruggen, M. Ashworth, S.T. LoDuca, C. Vlaeminck, E. Cocquyt, T. Sauvage, F.W. Zechman, D.S. Littler, M.M. Littler, F. Leliaert, O. De Clerck, A multi-locus time-calibrated phylogeny of the siphonous green algae, Mol. Phylogenet. Evol. 50 (2009) 642–653.

[26] I. Glaub, K. Vogel, Others, The stratigraphic record of microborings, Fossils and Strata. 51 (2004) 126–135.

[27] G. Radtke, M. Gektidis, S. Golubic, K. Hofmann, T. Le Campion-Alsumard, The identity of an endolithic alga: Ostreobium brabantium Weber-van Bosse is recognized as carbonate-penetrating rhizoids of Acetabularia (Chlorophyta, Dasycladales), CFS Courier Forschungsinstitut Senckenberg. (1997) 341–347.

[28] M.E. Bornet, C. Flahault, Sur Quelques Plantes Vivant Dans Le Test Calcaire Des Mollusques, Bulletin de La Société Botanique de France. 36 (1889) CXLVII–CLXXVI.

[29] K.J. Lukas, Two species of the chlorophyte genus Ostreobium from skeletons of Atlantic and Caribbean reef corals1, 2, J. Phycol. 10 (1974) 331–335.

[30] D.R. Kobluk, M.J. Risk, Algal borings and framboidal pyrite in Upper Ordovician brachiopods, Lethaia. 10 (1977) 135–143. https://doi.org/10.1111/j.1502-3931.1977.tb00602.x.

[31] M. Wisshak, M. Gektidis, A. Freiwald, T. Lundälv, Bioerosion along a bathymetric gradient in a cold-temperate setting (Kosterfjord, SW Sweden): an experimental study, Facies. 51 (2005) 93–117.

[32] M. Gektidis, Z. Dubinsky, S. Goffredo, Microendoliths of the Shallow Euphotic Zone in open and shaded habitats at 30°N – Eilat, Israel – paleoecological implications, Facies. 53 (2007) 43–55.

[33] E. Gutner-Hoch, M. Fine, Genotypic diversity and distribution of Ostreobium quekettii within scleractinian corals, Coral Reefs. 30 (2011) 643–650.

[34] J. del Campo, J.-F. Pombert, J. Šlapeta, A. Larkum, P.J. Keeling, The 'other' coral symbiont: Ostreobium diversity and distribution, ISME J. 11 (2016) 296–299.

[35] V.R. Marcelino, H. Verbruggen, Multi-marker metabarcoding of coral skeletons reveals a rich microbiome and diverse evolutionary origins of endolithic algae, Sci. Rep. 6 (2016) 31508.

[36] T. Sauvage, W.E. Schmidt, S. Suda, S. Fredericq, A metabarcoding framework for facilitated survey of endolithic phototrophs with tufA, BMC Ecol. 16 (2016) 8.

[37] A. Massé, I. Domart-Coulon, S. Golubic, D. Duché, A. Tribollet, Early skeletal colonization of the coral holobiont by the microboring Ulvophyceae Ostreobium sp, Sci. Rep. 8 (2018) 1–11.

[38] B. Koehne, G. Elli, R.C. Jennings, C. Wilhelm, H. Trissl, Spectroscopic and molecular characterization of a long wavelength absorbing antenna of Ostreobium sp, Biochim. Biophys. Acta. 1412 (1999) 94–107.

[39] C. Iha, K.E. Dougan, J.A. Varela, V. Avila, C.J. Jackson, K.A. Bogaert, Y. Chen, L.M. Judd, R. Wick, K.E. Holt, M.M. Pasella, F. Ricci, S.I. Repetti, M. Medina, V.R. Marcelino, C.X. Chan, H. Verbruggen, Genomic adaptations to an endolithic lifestyle in the coral-associated alga Ostreobium, Curr. Biol. (2021) 2020.07.21.211367. https://doi.org/10.1016/j.cub.2021.01.018.

[40] J.A. West, H. Calumpong, New records of marine algae from the Philippines, Micronesica. 23 (1990) 181–190.

[41] P. Kornmann, P.-H. Sahling, Ostreobium quekettii (Codiales, Chlorophyta), Helgoländer Meeresuntersuchungen. 34 (1980) 115–122.

[42] V. R Marcelino, M.C.M. Cremen, C.J. Jackson, A.A.W. Larkum, H. Verbruggen, Evolutionary Dynamics of Chloroplast Genomes in Low Light: A Case Study of the Endolithic Green Alga Ostreobium quekettii, Genome Biol. Evol. 8 (2016) 2939–2951.

[43] M.C.M. Cremen, F. Leliaert, V.R. Marcelino, H. Verbruggen, Large Diversity of Nonstandard Genes and Dynamic Evolution of Chloroplast Genomes in Siphonous Green Algae (Bryopsidales, Chlorophyta), Genome Biol. Evol. 10 (2018) 1048–1061.

[44] S.I. Repetti, C.J. Jackson, L.M. Judd, R.R. Wick, K.E. Holt, H. Verbruggen, The inflated mitochondrial genomes of siphonous green algae reflect processes driving expansion of noncoding DNA and proliferation of introns, PeerJ. 8 (2020) e8273.

[45] F. Leliaert, D.R. Smith, H. Moreau, M.D. Herron, H. Verbruggen, C.F. Delwiche, O. De Clerck, Phylogeny and Molecular Evolution of the Green Algae, CRC Crit. Rev. Plant Sci. 31 (2012) 1–46.

[46] F. Zheng, H. Liu, M. Jiang, Z. Xu, Z. Wang, C. Wang, F. Du, Z. Shen, B. Wang, The complete mitochondrial genome of the Caulerpa lentillifera (Ulvophyceae, Chlorophyta): Sequence, genome content, organization structure and phylogenetic consideration, Gene. 673 (2018) 225–238.

[47] O. De Clerck, S.-M. Kao, K.A. Bogaert, J. Blomme, F. Foflonker, M. Kwantes, E. Vancaester, L. Vanderstraeten, E. Aydogdu, J. Boesger, G. Califano, B. Charrier, R. Clewes, A. Del Cortona, S. D'Hondt, N. Fernandez-Pozo, C.M. Gachon, M. Hanikenne, L. Lattermann, F. Leliaert, X. Liu, C.A. Maggs, Z.A. Popper, J.A. Raven, M. Van Bel, P.K.I. Wilhelmsson, D. Bhattacharya, J.C. Coates, S.A. Rensing, D. Van Der Straeten, A. Vardi, L. Sterck, K. Vandepoele, Y. Van de Peer, T. Wichard, J.H. Bothwell, Insights into the Evolution of Multicellularity from the Sea Lettuce Genome, Curr. Biol. 28 (2018) 2921-2933.e5.

[48] A. Arimoto, K. Nishitsuji, Y. Higa, N. Arakaki, K. Hisata, C. Shinzato, N. Satoh, E. Shoguchi, A siphonous macroalgal genome suggests convergent functions of homeobox genes in algae and land plants, DNA Res. 26 (2019) 183–192.

[49] W.F. Prud'homme van Reine, C. Van Den Hoek, Isolation of living Algae growing in the shells of Molluscs and Barnacles with EDTA (ethylenediaminetetraacetic acid), Blumea: Biodiversity, Evolution and Biogeography of Plants. 14 (1966) 331–332.

[50] M. Wilkinson, Investigations on the autecology of Eugomontia sacculata Kornm., A shell-boring alga, J. Exp. Mar. Bio. Ecol. 16 (1974) 19–27.

[51] R.A. Andersen, Algal Culturing Techniques, Elsevier, 2005.

[52] P.J. Harrison, J.A. Berges, Marine Culture Media, Algal Culturing Techniques. (2005) 21–33. https://doi.org/10.1016/b978-012088426-1/50004-4.

[53] J. Hollants, O. Leroux, F. Leliaert, H. Decleyre, O. De Clerck, A. Willems, Who is in there? Exploration of endophytic bacteria within the siphonous green seaweed Bryopsis (Bryopsidales, Chlorophyta), PLoS One. 6 (2011) e26458.

[54] T. Aires, Y. Moalic, E.A. Serrao, S. Arnaud-Haond, Hologenome theory supported by cooccurrence networks of species-specific bacterial communities in siphonous algae (Caulerpa), FEMS Microbiol. Ecol. 91 (2015). https://doi.org/10.1093/femsec/fiv067.

[55] G.H. Kim, T.A. Klotchkova, Y.M. Kang, Life without a cell membrane: regeneration of protoplasts from disintegrated cells of the marine green alga Bryopsis plumosa, J. Cell Sci. 114 (2001) 2009–2014.

[56] G.H. Kim, L. Fritz, A SIGNAL GLYCOPROTEIN WITH α-d-MANNOSYL RESIDUES IN INVOLVED IN THE WOUND-HEALING RESPONSE OF ANTITHAMNION SPARSUM (CREAMIALES, RHODOPHYTA)1, J. Phycol. 29 (1993) 85–90.

[57] G.H. Kim, I.K. Lee, L. Fritz, The wound-healing responses of Antithamnion nipponicum and Griffithsia pacifica (Ceramiales, Rhodophyta) monitored by lectins, Phycological Res. 43 (1995) 161–166.

[58] M.G. Jung, K.P. Lee, H.-G. Choi, S.-H. Kang, T.A. Klochkova, J.W. Han, G.H. Kim, Characterization of carbohydrate combining sites of Bryohealin, an algal lectin from Bryopsis plumosa, J. Appl. Phycol. 22 (2010) 793–802.

[59] C.J. O’Kelly, C. Yarish, Observations on marine chaetophoraceae (Chlorophyta). I. Sporangial ontogeny in the type species of Entocladia and Phaeophila 1, J. Phycol. 16 (1980) 549–558.

[60] C.J. O’Kelly, B. Wysor, W.K. Bellows, Collinsiella (Ulvophyceae, Chlorophyta) and other ulotrichalean taxa with shell-boring sporophytes form a monophyletic clade, Phycologia. 43 (2004) 41–49.

[61] A. Shinohara, M. Shinohara, Roles of RecA homologues Rad51 and Dmc1 during meiotic recombination, Cytogenet. Genome Res. 107 (2004) 201–207.

[62] K.E. Clifton, L.M. Clifton, The phenology of sexual reproduction by green algae (Bryopsidales) on Caribbean coral reefs, J. Phycol. 35 (1999) 24–34.

[63] A.M. Schurko, J.M. Logsdon Jr, Using a meiosis detection toolkit to investigate ancient asexual “scandals” and the evolution of sex, Bioessays. 30 (2008) 579–589.

[64] E. Calderón-Sáenz, R. Schnetter, Life Cycle and Morphology ofBryopsidella ostreobiformisspec. nov. (Bryopsidaceae, Chlorophyta) from the Mediterranean, under Culture Conditions, with Comments on the Phylogeny of theBryopsis/DerbesiaComplex, Bot. Acta. 102 (1989) 249–260.

[65] M.M. Littler, D.S. Littler, S.M. Blair, J.N. Norris, Deep-water plant communities from an uncharted seamount off San Salvador Island, Bahamas: distribution, abundance, and primary productivity, Deep Sea Res. A. 33 (1986) 881–892.

[66] J.W. Kanwisher, S.A. Wainwright, OXYGEN BALANCE IN SOME REEF CORALS, Biol. Bull. 133 (1967) 378–390.

[67] P. Halldal, PHOTOSYNTHETIC CAPACITIES AND PHOTOSYNTHETIC ACTION SPECTRA OF ENDOZOIC ALGAE OF THE MASSIVE CORAL FAVIA, Biol. Bull. 134 (1968) 411–424.

[68] S.H. Magnusson, M. Fine, M. Kühl, Light microclimate of endolithic phototrophs in the scleractinian corals Montipora monasteriata and Porites cylindrica, Mar. Ecol. Prog. Ser. 332 (2007) 119–128.

[69] N. Shashar, N. Stambler, Endolithic algae within corals - life in an extreme environment, Journal of Experimental Marine Biology and Ecology. 163 (1992) 277–286. https://doi.org/10.1016/0022-0981(92)90055-f.

[70] P.J. Ralph, A.W.D. Larkum, M. Kühl, Photobiology of endolithic microorganisms in living coral skeletons: 1. Pigmentation, spectral reflectance and variable chlorophyll fluorescence analysis of endoliths in the massive corals Cyphastrea serailia, Porites lutea and Goniastrea australensis, Mar. Biol. 152 (2007) 395–404.

[71] C. Wilhelm, T. Jakob, Uphill energy transfer from long-wavelength absorbing chlorophylls to PS II in Ostreobium sp. is functional in carbon assimilation, Photosynth. Res. 87 (2006) 323–329.

[72] D.C. Fork, A.W.D. Larkum, Light harvesting in the green alga Ostreobium sp., a coral symbiont adapted to extreme shade, Mar. Biol. 103 (1989) 381–385.

[73] M. Kühl, G. Holst, A.W.D. Larkum, P.J. Ralph, Imaging of oxygen dynamics within the endolithic algal community of the massive coral Porites Lobata(1), J. Phycol. 44 (2008) 541–550.

[74] M. Chen, M. Schliep, R.D. Willows, Z.-L. Cai, B.A. Neilan, H. Scheer, A red-shifted chlorophyll, Science. 329 (2010) 1318–1319.

[75] K. Oshita, T. Suzuki, T. Kawano, Possible roles of near-infrared light on the photosynthesis in Synechocystis sp. PCC6803 under solar simulating artificial light, Environ. Control. Biol. 56 (2018) 17–21.

[76] M. Kühl, M. Chen, P.J. Ralph, U. Schreiber, A.W.D. Larkum, Ecology: a niche for cyanobacteria containing chlorophyll d, Nature. 433 (2005) 820.

[77] T. Friedrich, F.-J. Schmitt, Red-Shifted and Red Chlorophylls in Photosystems: Entropy as a Driving Force for Uphill Energy Transfer?, in: J.-R. Shen, K. Satoh, S.I. Allakhverdiev (Eds.), Photosynthesis: Molecular Approaches to Solar Energy Conversion, Springer International Publishing, Cham, 2021: pp. 247–275.

[78] M. Kühl, E. Trampe, M. Mosshammer, M. Johnson, A.W. Larkum, N.-U. Frigaard, K. Koren, Substantial near-infrared radiation-driven photosynthesis of chlorophyll f-containing cyanobacteria in a natural habitat, Elife. 9 (2020). https://doi.org/10.7554/eLife.50871.

[79] T. Morosinotto, S. Castelletti, J. Breton, R. Bassi, R. Croce, Mutation analysis of Lhca1 antenna complex. Low energy absorption forms originate from pigment-pigment interactions, J. Biol. Chem. 277 (2002) 36253–36261.

[80] T. Morosinotto, J. Breton, R. Bassi, R. Croce, The nature of a chlorophyll ligand in Lhca proteins determines the far red fluorescence emission typical of photosystem I, J. Biol. Chem. 278 (2003) 49223–49229.

[81] C. Six, A.Z. Worden, F. Rodríguez, H. Moreau, F. Partensky, New insights into the nature and phylogeny of prasinophyte antenna proteins: Ostreococcus tauri, a case study, Mol. Biol. Evol. 22 (2005) 2217–2230.

[82] M. Fine, E. Meroz-Fine, O. Hoegh-Guldberg, Tolerance of endolithic algae to elevated temperature and light in the coral Montipora monasteriata from the southern Great Barrier Reef, J. Exp. Biol. 208 (2005) 75–81.

[83] R. Hill, C.M. Brown, K. DeZeeuw, D.A. Campbell, P.J. Ralph, Increased rate of D1 repair in coral symbionts during bleaching is insufficient to counter accelerated photo-inactivation, Limnol. Oceanogr. 56 (2011) 139–146.

[84] W. Wang, X. Qin, M. Sang, D. Chen, K. Wang, R. Lin, C. Lu, J.-R. Shen, T. Kuang, Spectral and functional studies on siphonaxanthin-type light-harvesting complex of photosystem II from Bryopsis corticulans, Photosynth. Res. 117 (2013) 267–279.

[85] V. Giovagnetti, G. Han, M.A. Ware, P. Ungerer, X. Qin, W.-D. Wang, T. Kuang, J.-R. Shen, A.V. Ruban, A siphonous morphology affects light-harvesting modulation in the intertidal green macroalga Bryopsis corticulans (Ulvophyceae), Planta. 247 (2018) 1293–1306.

[86] A. Tribollet, C. Langdon, S. Golubic, M. Atkinson, ENDOLITHIC MICROFLORA ARE MAJOR PRIMARY PRODUCERS IN DEAD CARBONATE SUBSTRATES OF HAWAIIAN CORAL REEFS1, Journal Of. 42 (2006) 292–303.

[87] J. Schneider, H. Torunski, Biokarst on limestone coasts, morphogenesis and sediment production, Mar. Ecol. 4 (1983) 45–63.

[88] F. Garcia-Pichel, Plausible mechanisms for the boring on carbonates by microbial phototrophs, Sediment. Geol. 185 (2006) 205–213.

[89] F. Garcia-Pichel, E. Ramírez-Reinat, Q. Gao, Microbial excavation of solid carbonates powered by P-type ATPase-mediated transcellular Ca2+ transport, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 21749–21754.

[90] B.S. Guida, F. Garcia-Pichel, Extreme cellular adaptations and cell differentiation required by a cyanobacterium for carbonate excavation, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 5712–5717.

[91] A. Tribollet, A. Chauvin, P. Cuet, Carbonate dissolution by reef microbial borers: a biogeological process producing alkalinity under different pCO2 conditions, Facies. 65 (2019). https://doi.org/10.1007/s10347-018-0548-x.

[92] E.A. Drew, K.M. Abel, Studies on Halimeda IV. An endogenous rhythm of chloroplast migration in the siphonous green alga, H. distorta, J. Interdiscipl. Cycle Res. 23 (1992) 128–135.

[93] C. Godinot, A. Tribollet, R. Grover, Bioerosion by euendoliths decreases in phosphate-enriched skeletons of living corals, (2012). https://bg.copernicus.org/articles/9/2377/2012/.

[94] V.R. Marcelino, K.M. Morrow, M.J.H. van Oppen, D.G. Bourne, H. Verbruggen, Diversity and stability of coral endolithic microbial communities at a naturally high pCO2 reef, Mol. Ecol. 26 (2017) 5344–5357.

[95] A.J. Fordyce, T.D. Ainsworth, W. Leggat, Light Capture, Skeletal Morphology, and the Biomass of Corals’ Boring Endoliths, MSphere. 6 (2021). https://doi.org/10.1128/mSphere.00060-21.

[96] E.A. Titlyanov, T.V. Titlyanova, D.J. Chapman, Dynamics and patterns of algal colonization on mechanically damaged and dead colonies of the coral Porites lutea, 51 (2008) 285–296.

[97] C.J. Bentis, L. Kaufman, S. Golubic, Endolithic fungi in reef-building corals (Order : Scleractinia) are common, cosmopolitan, and potentially pathogenic, Biol. Bull. 198 (2000) 254–260.

[98] F.L. Gonzalez-Zapata, S. Gómez-Osorio, J.A. Sánchez, Conspicuous endolithic algal associations in a mesophotic reef-building coral, Coral Reefs. 37 (2018) 705–709.

Table 1. Publicly available resources of *Ostreobium*

Strain	Origin	Public Repository (Voucher #)	Molecular Resources				References
			Molecular markers	*Nuclear genome*	*Chloroplast genome*	*Mitochondrial*	
SAG 6.99	Epiphytic on red alga (Philippines)	SAG (6.99)	Available from the complete genome	PRJEB35267	LT593849	MN514984.1	West & Calumpong 1990 Marcelino *et al.* 2016 Repetti *et al.* 2020 Iha *et al.* 2021
VRM605	Endolithic in coral (Great Barrier Reef)	ANACC (CS-1379)	Available from the complete chloroplast genome	NA	OK189523	NA	Pasella *et al.* 2021
VRM609	Endolithic in coral (Great Barrier Reef)	ANACC (CS-1380)	Available from the complete chloroplast genome	NA	OK189524	NA	Pasella *et al.* 2021
VRM623	Endolithic in coral (Great Barrier Reef)	ANACC (CS-1381)	NA	NA	NA	NA	Pasella *et al.* 2021
VRM627	Endolithic in coral (Great Barrier Reef)	ANACC (CS-1382)	Available from the complete chloroplast genome	NA	OK189525	NA	Pasella *et al.* 2021
VRM633	Endolithic in coral (Great Barrier Reef)	ANACC (CS-1383)	Available from the complete chloroplast genome	NA	OK189526	NA	Pasella *et al.* 2021
VRM638	Endolithic in coral (Great Barrier Reef)	ANACC (CS-1384)	NA	NA	NA	NA	Pasella *et al.* 2021
VRM642	Endolithic in coral (Great	ANACC (CS-1385)	Available from the complete	NA	OK189527	NA	Pasella *et al.* 2021

	Barrier Reef)		chloroplast genome				
VRM644	Endolithic in coral (Great Barrier Reef)	ANACC (CS-1386)	Available from the complete chloroplast genome	NA	OK189528	NA	Pasella *et al.* 2021
VRM646	Endolithic in coral (Great Barrier Reef)	ANACC (CS-1387)	Available from the complete chloroplast genome	NA	OK189529	NA	Pasella *et al.* 2021
VRM647	Endolithic in coral (Great Barrier Reef)	NA	Available from the complete chloroplast genome	NA	OK189530	NA	Pasella *et al.* 2021
VRM650	Endolithic in coral (Great Barrier Reef)	ANACC (CS-1388)	Available from the complete chloroplast genome	NA	OK189531	NA	Pasella *et al.* 2021
010	Endolithic in coral (Aquarium Tropical du Palais de la Porte Dorée)	MNHN RBCell (MNHN-ALCP-2019-873.3)	*rbc*L (MK095212)	NA	NA	NA	Masse *et al.* 2020
017	Endolithic in coral (Aquarium Tropical du Palais de la Porte Dorée)	MNHN RBCell (MNHN-ALCP-2019-873.4)	*rbc*L (MK095214)	NA	NA	NA	Masse *et al.* 2020
018B	Endolithic in coral (Aquarium Tropical du Palais de la Porte Dorée)	MNHN RBCell (MNHN-ALCP-2019-873.6)	*rbc*L (MK095215)	NA	NA	NA	Masse *et al.* 2020
019	Endolithic in coral (Aquarium Tropical du Palais de la Porte Dorée)	MNHN RBCell (MNHN-ALCP-2019-873.8)	*rbc*L (MK095213)	NA	NA	NA	Masse *et al.* 2020

05	Endolithic in coral (Aquarium Tropical du Palais de la Porte Dorée)	MNHN RBCell (MNHN-ALCP-2019-873.1)	*rbc*L (MK095217)	NA	NA	NA	Masse *et al.* 2020
018C	Endolithic in coral (Aquarium Tropical du Palais de la Porte Dorée)	MNHN RBCell (MNHN-ALCP-2019-873.7)	*rbc*L (MK095216)	NA	NA	NA	Masse *et al.* 2020
022	Endolithic in coral (Aquarium Tropical du Palais de la Porte Dorée)	NA	*rbc*L (MK095219)	NA	NA	NA	Masse *et al.* 2020
018A	Endolithic in coral (Aquarium Tropical du Palais de la Porte Dorée)	MNHN RBCell (MNHN-ALCP-2019-873.5)	*rbc*L (MK095218)	NA	NA	NA	Masse *et al.* 2020
06	Endolithic in coral (Aquarium Tropical du Palais de la Porte Dorée)	MNHN RBCell (MNHN-ALCP-2019-873.2)	*rbc*L (MK095220)	NA	NA	NA	Masse *et al.* 2020