



Beyond the Symbiodiniaceae: diversity and role of microeukaryotic coral symbionts

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Abstract Many corals form intimate symbioses with photosynthetic dinoflagellates in the family Symbiodiniaceae. These symbioses have been deeply studied, particularly in reef-forming corals. The complex microbial community that is associated with corals contains other members that have also been well characterized such as bacteria. However, our understanding of the coral holobiont and subsequently coral reef ecosystems is not complete if we do not take into consideration the microeukaryotes like protists and fungi. Microeukaryotes are currently the greatest enigma within the coral microbiome. Only a handful of them have been characterized, very few have been cultured and even less have genomes available. This is a reflection of a smaller community of scientists working on this particular group of organisms when compared with bacteria or Symbiodiniaceae, but also of the many technical challenges that we face when trying to study microeukaryotes. Recent advances in the use of metabarcoding are revealing the importance of microeukaryotes in corals in terms of abundance and presence, with notable examples being the green algae *Ostreobium* and the

apicomplexans Corallicolidae. We believe that it is timely and necessary to present what we know so far about coral microeukaryotes before the expected flow of high-throughput metabarcoding studies exploring the microeukaryotic fraction of the coral microbiome.

Keywords Holobiont · Microbiome · Symbionts · Microeukaryotes · Protists · Fungi · Culturing · Metabarcoding

Introduction

Corals reefs support hyper-diverse ecosystems and build natural offshore structures that protect our coastlines from storm surge and coastal inundation (Reaka-Kudla et al. 1997; Harris et al. 2018). The corals that build these structures are able to do so by forming intimate symbioses with dinoflagellates in the family Symbiodiniaceae, which provide photosynthetically fixed carbon to the coral host as an energy source (Schlichter et al. 1995). Apart from their photosynthetic symbiotic algae, corals are known to host a complex microbial community. The coral and all its microbial partners are known as the coral holobiont (Rohwer et al. 2002). Some elements of the holobiont have been studied in detail such as the aforementioned Symbiodiniaceae or bacteria. However, the microeukaryotes (microscopic fungi and protists) associated with corals have been less studied, limiting our capacity to fully understand the coral holobiont and subsequently the coral reef ecosystem (Ainsworth et al. 2017). The most prevalent approaches to study the holobiont are ‘molecular techniques’, principally those based on DNA and RNA sequencing. Among those methods, metabarcoding is the most common one used to explore the biodiversity within the holobiont (Rohwer et al. 2002). Even today

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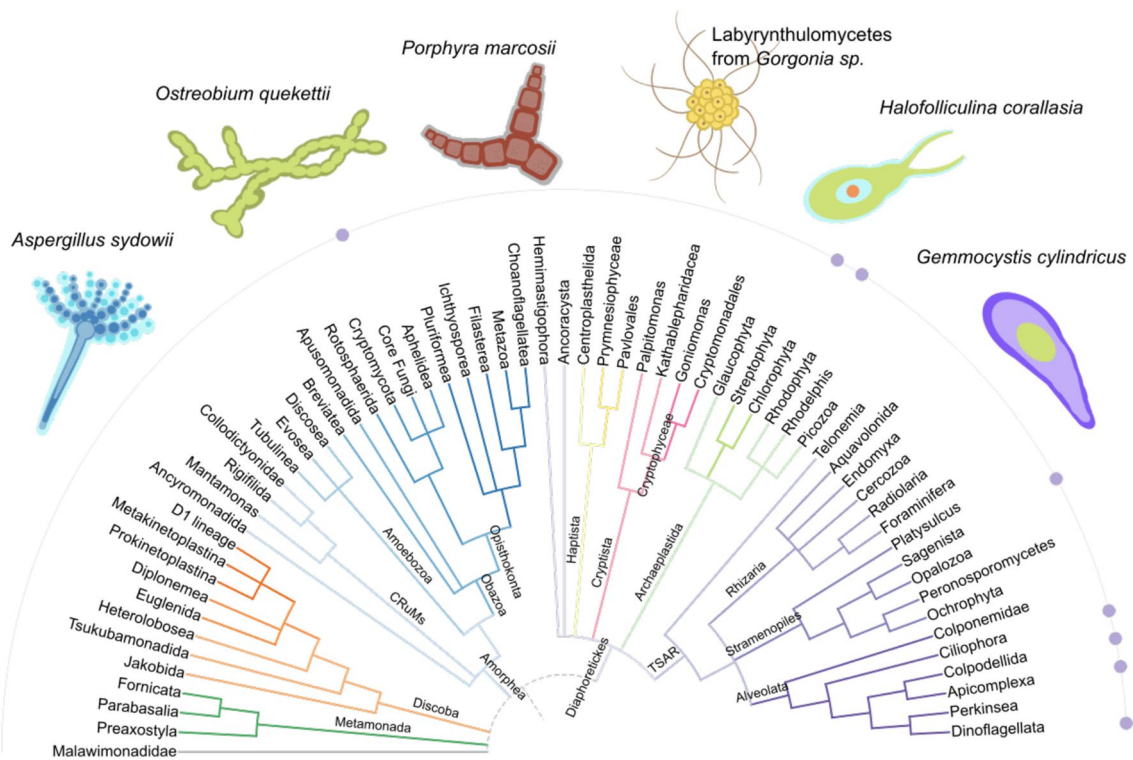


Fig. 1 Eukaryotic tree showing the groups that contain microeukaryotes that have been isolated from corals (purple dots). Some examples of these organisms are presented above the tree. Tree based on EukProt (Richter et al. 2022)

microeukaryotes tend to be an overlooked, if not forgotten, part of most microbiome studies. This largely reflects the challenges that culturing microeukaryotes presents as compared to bacteria and also the technical limitations in the use of metabarcoding approaches that preclude an extensive exploration of the coral microeukaryote community (Keeling and del Campo 2017; del Campo et al. 2020).

Identifying and assigning roles or functions to all microbial components of coral microbiomes, from viruses to microeukaryotes, is key for the understanding of coral biology (van Oppen and Blackall 2019). Here, we will summarize the diversity of microeukaryotes (beyond Symbiodiniaceae) that have been identified in association with corals so far, mostly through isolation and microscopical characterization (Fig. 1). As well, we propose solutions to the limitations we currently have to exploring this particular component of the coral holobiont.

Phototrophic microeukaryotes

The Symbiodiniaceae dinoflagellates are the best studied microbial members of the coral holobiont. That said, they have only recently been characterized properly and described to the species level (LaJeunesse et al. 2018). However, Symbiodiniaceae are not the only algae known to be associated

with corals. Algae are key players in coral reef ecosystems. For example in temperate reefs, calcifying algae are dominant instead of reef building corals (Ballesteros 2006). As well, algae compete for space and resources with the corals in tropical reefs, and since both are sessile they are in permanent competition and biochemical “warfare” (Roach et al. 2020). As a result, most algae other than Symbiodiniaceae are commonly considered coral foes, competitors, or bioeroders of coral skeletons. In recent years, however, some of these relationships have been re-evaluated, in particular green and red algae, which are most commonly reported in direct association with corals.

Chlorophyta

The chlorophytes, or green algae, are photosynthetic eukaryotes with double membrane-bound plastids containing chlorophyll *a* and *b*. They are ubiquitous in aquatic habitats and have a variety of morphologies and lifestyles ranging from free-swimming unicellular forms (e.g., *Chlamydomonas* or *Micromonas*) to macroscopic benthic forms (e.g., *Ulva* or *Caulerpa*) (Lewis and McCourt 2004). The chlorophyte lineage is typically split into four classes: the Chlorophyceae and Trebouxiophyceae are both well-supported clades by molecular phylogenies,

while the Prasinophyceae and Ulvophyceae are traditional classes which are not supported as monophyletic clades in molecular phylogenies. The Ulvophyceae are very diverse (Leliaert et al. 2012) and stand out because they include many microbial members but are more typically found as large, macroscopic forms. *Ostreobium sp.* (Bryopsidales, Ulvophyceae) have been shown to be a pervasive member of the endolithic community of coral (Tandon et al. 2022). This makes them unique among the chlorophytes in that they thrive in the low-light and variable pH and O₂ environment of the coral skeleton where they are often seen as a visible green ring just below the coral tissue. They have also been shown to interact with prokaryotes like the cyanobacteria *Halomiconema* (Williams et al. 2015). *Ostreobium* are capable of exchanging nitrogen and carbon with the coral and are most likely horizontally transmitted from adult to recruit (Massé et al. 2018). Genomic evidence now suggests that *Ostreobium* supplements their carbon needs by feeding on the organic skeletal matrix of the coral host while relying upon other (likely bacterial) holobiont members for vitamin B12 (Iha et al. 2021). Considering it shares similar biogeographical patterns and distinct subclasses with host-species preference, like Symbiodiniaceae, *Ostreobium* has been hypothesized to have co-evolved with corals and Symbiodiniaceae (del Campo et al. 2017). However, whether *Ostreobium* are indeed beneficial to the coral or a parasite of the coral remains to be confirmed (Verbruggen and Tribollet 2011). The former seems most likely given it absorbs light at a wavelength not used by Symbiodiniaceae and provides photosynthates to the coral (Schlichter et al. 1995). It has even been suggested that *Ostreobium* can replace the Symbiodiniaceae function during bleaching events and temporarily sustain the coral prior to a possible return of the Symbiodiniaceae (Fine and Loya 2002). Notably, recent metatranscriptomics work has shown *Ostreobium* blooms to become the dominant oxygenic phototroph of the coral holobiont during bleaching (Iha et al. 2021). As well, according to the most recent research on *Ostreobium*, the endolithic algae can contribute to coral recovery after bleaching when it blooms near the skeletal surface, alleviating the high light stress of the bleached corals by reducing skeleton reflectance (Galindo-Martínez et al. 2022). Besides *Ostreobium*, a few other chlorophytes have been recovered from coral holobionts, including *Phaeophila sp.* (Phaeophilales, Ulvophyceae), *Ochlochaete sp.* (Ulvales, Ulvophyceae), *Ulvella sp.* (Ulvales, Ulvophyceae), and several unidentified Cladophorales (Marcelino and Verbruggen 2016). *Phaeophila* is a known microborer, while *Ulvella* has been associated with pathogenesis in some corals (Goldberg et al. 1984). Despite these advances in our knowledge, the

full diversity of chlorophytes and their potential role in the coral holobiont is still unclear.

Rhodophytes

The Rhodophyta, or red algae, are a highly diverse group of eukaryotic algae which cells lack flagella and centrioles. Their chloroplasts contain phycobilins which give them their characteristic red color; they also contain other pigments such as chlorophyll *a* (Yoon et al. 2006). Although they primarily live in marine environments, ranging from deep oceanic depths to shallow intertidal zones, they are known to flourish in freshwater and on land (Yoon et al. 2016). Remarkably the plastids of apicomplexans and dinoflagellates, both of which have tight associations with cnidarians, originated from a common red algal endosymbiont (Janouškovec et al. 2010)—a fact alone that seals the importance of the red algae to the health of coral reefs. Rhodophytes are ecologically important in their own right as primary producers and habitat structurers. Today, the best known rhodophytes on the reef are the crustose coralline algae (CCA). Certain CCA have been identified as primary inducers of metamorphosis for coral larvae (Morse et al. 1988). Some species of CCA that have been shown to induce metamorphosis include *Lithophyllum insispidium*, *Hydrolithon onkodes*, *Neogoniolithon brassica*, *Mesophyllum sp.*, and *Penyssonnelia sp.* (Heyward and Negri 1999). However, the relationship is not always so straight forward and coral larvae face a battle with the CCA and try not to be overgrown during the first few weeks of development (Craggs et al. 2019). Like corals, the CCA appear to have their own distinct microbiome with specific bacteria that reside within the algae and appear to be paramount to the facilitation of metamorphosis in the coral larvae (Webster et al. 2004). Beyond their role in coral settlement, rhodophytes have been found in abundance within the coral's microbiome. Similar to *Ostreobium* they often reside in the coral skeleton. This association was first identified in 1995, when Le Campion-Alsumard and colleagues highlighted conchocelis life stages of rhodophytes within corals. They noted how the presence of the conchocelis filaments appeared to exclude other endolithic algae in the skeleton, such as *Ostreobium* (Le Campion-Alsumard et al. 1995). Red algae within coral microbiomes were then mostly overlooked until 2016, when Pica et al. identified the conchocelis stage of the rhodophyte *Porphyra* to be the most abundant endolith within stylasterid hydrozoans (Pica et al. 2016). Stylasterids are tropical azooxanthellate lace corals with unusual coloration ranging from red to purple on the photophilic portions of the colony. When researchers dug a little deeper, they found that the calcareous skeletons of stylasterids are most likely invaded and eroded by the conchocelis stages of bangialean rhodophytes, such as *Porphyra*

marcosii and *Pyropia vietnamensis* (Tribollet et al. 2018). Whether these rhodophytes confer any advantage to the coral host remains to be uncovered.

Heterotrophic microeukaryotes

Heterotrophic protists and fungi have historically been associated with coral disease. The most notorious of these would be the fungi *Aspergillus sydowii* which for a long time was thought to be responsible for the massive Caribbean sea fan mortalities at the end of the nineties (Sweet et al. 2012). Other well-known microeukaryotic pathogens of corals are the ciliate *Halofolliculina corallasia* which is responsible for eroding band syndrome (AKA Caribbean ciliate infection) and *Philaster guamensis* and *Philaster lucinda* which are involved in the pathogenesis of white syndrome, white band disease and brown band disease (Sweet and Séré 2016). As the focus has primarily been on disease, the general impression is that most heterotrophic microeukaryotes associated with corals are pathogenic, but less targeted approaches have led to a more complicated picture. Certain protists that could be easily suspected of being parasites, such as apicomplexans, are now known to be widespread across both coral geographic distribution and taxonomic diversity, and are present in apparently healthy coral colonies while not being associated with any disease (Kwong et al. 2019). In addition, molecular sampling of the diversity of eukaryotes in coral communities shows a broad range of heterotrophic eukaryotes associated with corals, interestingly, many of which are alveolates (Brenner-Raffalli et al. 2018; del Campo et al. 2019).

Apicomplexans and related lineages

Apicomplexans are a large group of parasitic alveolates that cause devastating human diseases such as malaria. These parasites evolved from photosynthetic ancestors and retained a relict non-photosynthetic plastid. Some close relatives, called chromerids, are still photosynthetic, and the only two chromerids that have been formally described, *Chromera velia* and *Vitrella brassicaformis*, were both isolated from coral reefs (Moore et al. 2008; Oborník et al. 2012). That the photosynthetic relatives of apicomplexans are somehow linked to coral reefs has prompted a major re-evaluation of the ecological conditions and symbiotic associations that drove the evolution of parasitism (Janouškovec et al. 2015; Woo et al. 2015). However, the chromerids relationship with corals has been disputed. Chromerids appear to be residents of the biogenous sediment of coral reefs rather than part of the coral holobiont and perhaps only live with or in the actual coral for limited periods of time (Mathur et al. 2018). Historically, corals have also not been considered a common

host for apicomplexans: sporadic reports in the last 30 yrs include a morphological description of a single coccidian, *Gemmocystis cylindrus* (Upton and Peters 1986), the detection of apicomplexan 18S rRNA gene sequences (called “Type-N”) (Toller et al. 2002; Šlapeta and Linares 2013), and plastid 16S rRNA gene of uncharacterized Apicomplexan-Related Lineages (ARLs) (Janouškovec et al. 2012, 2013). In a recent study, Type-N and ARL-V were proven to be the same group of organisms, and structurally similar to *G. cylindricus* (Kwong et al. 2019). These symbionts, informally known as corallicolids, are widespread across corals (in some cases as abundant as Symbiodiniaceae), and although the corallicolid plastid is non-photosynthetic, it nevertheless retains genes for chlorophyll biosynthesis (Kwong et al. 2019). Electron microscopy further confirmed that these symbionts live intracellularly within the gastrodermis of coral hosts, just like Symbiodiniaceae. In 2021, the Corallicolida order was officially designated with *Corallicola aquarius* as the type species. All members of the clade, which now includes the reassigned *G. cylindrus* along with *Anthozoaphila gnarlus*, are exclusively found in association with Anthozoans (Kwong et al. 2021). The ubiquity of these general anthozoan symbionts is further emphasized by the finding that corallicolids can be associated with coral hosts at depths of 1400 m (Vohsen et al. 2020). Corallicolids and chromerids are promising candidates in which to study the evolutionary transition to parasitism in apicomplexans and how the loss of photosynthesis affected this process.

Fungi

The kingdom Fungi encompasses diverse forms of saprotrophs, including both unicellular and multicellular taxa that can reproduce both sexually (spores) and asexually. Fungi present a cell wall made of β -glucan and usually chitin. The majority of aquatic fungi belong to two phyla, Ascomycota and Chytridiomycota, which are known to play critical functional roles within their ecosystems, for example infecting and consuming normally non-digestible phytoplankton (e.g., colonial diatoms), contributing organic matter to the microbial loop that would otherwise sink (Grossart and Rojas-Jimenez 2016). Fungi have been observed in association with corals, and are suggested to have both beneficial and saprophytic symbioses (Paulino et al. 2020). Beneficial ecological roles of the mycobiome (collective term of the fungal associates and their host) are thought to surround the heterotrophic conversion of reef biomass to nutrients (Morrison-Gardiner 2002). Saprophytic relationships are best known with the yeast *Aspergillus sydowii*, the causative agent of the Caribbean Sea fan disease (Smith et al. 1996; Alker et al. 2001; Troeger et al. 2014). *Aspergillus* has also been shown to be present in apparently healthy coral, suggesting that the ‘pathogenic’ nature of this fungi

might not be fully understood. Similarly, fungi including *Cladosporium* and *Fusarium* for example have been linked via both histological evidence and ITS sequences to a newly described disease in Micronesia – named gray patch disease (Sweet et al. 2013, 2019). Another fungal genus, *Rhytisma*, has been associated with dark spot syndrome in Caribbean corals (Sweet et al. 2013). That said, similar to *Aspergillus*, confirmation of pathogenesis is lacking for all these examples. Regardless of the exact role these fungi are playing in the coral holobiont, we do know that many boring endolithic fungi are associated with the coral skeleton (Golubic et al. 2005). However, it should be noted that although sequencing has highlighted the presence of these fungi only relatively few studies have attempted to culture the fungi and described those obtained to the species level (Kendrick et al. 1982; Bak and Laane 1987; Ravindran et al. 2001). Even though ITS-based analysis is a straightforward option for the characterization of fungi in corals (Schoch et al. 2012), there are issues with the sequencing specifically with regard to cross-amplification of the host and fungal cells—which likely underestimates the fungal diversity in any given sample (Amend et al. 2012; Bonthond et al. 2018; Góes-Neto et al. 2020; Paulino et al. 2020).

Ciliates

Ciliates are unicellular heterotrophic alveolates that inhabit nearly all environments on Earth, most as free-living organisms, but also including many that specialize as ecto/endo-symbionts of an animal host (Foissner et al. 2008). Free-living ciliates have even been suggested to play critical roles in the likes of marine trophic dynamics for example, linking primary producers and consumers (Gifford 1991). Characterized by their relatively complex body plan and the presence of hair-like organelles called cilia, these organisms have a wide range of morphologies that vary in sizes from 10 µm to a few millimeters (Foissner et al. 2008). Ciliates have been found to form associations with a wide variety of hosts, in many cases apparently pathogenic or opportunistic feeders on host tissues during pathogenesis (Sniezek et al. 1995; Poynton et al. 2001; Song et al. 2009). While there are a variety of ciliates observed in association with corals (Sweet and Séré 2016), they are rarely seen in healthy individuals. Instead, most ciliates are either associated with disease or found at the front of advancing lesions in diseased specimen (Katz et al. 2014; Sweet and Séré 2016), such as Caribbean ciliate infection (Cróquer et al. 2006a, 2006b; Rodríguez et al. 2009; Sweet et al. 2014; Sweet and Séré 2016; Montano et al. 2020), white band disease (Sweet et al. 2014), white plague disease (Randall et al. 2015), white syndrome (Sweet and Bythell 2012, 2015), skeletal eroding band disease (Antonius 1999; Antonius and Lipscomb 2001; Anthony et al. 2008; Montano et al. 2020), or brown

band disease (Bourne et al. 2008; Katz et al. 2014; Randall et al. 2015), and brown jelly syndrome (Sweet et al. 2012; Randall et al. 2015). Unfortunately, many early studies lack molecular surveys, a critical method to recover the diversity of the ciliate community. That said, the more recent work has described a number of ciliates present in corals and on a global scale (Sweet and Séré 2016). Ciliates of particular interest that have been observed in association with coral disease are the folliculinids (Cróquer et al. 2006b) and the scuticociliates (Bourne et al. 2008). *Halofolliculina coral-lasia* was the first protistan pathogen identified in corals and is responsible for skeletal eroding band syndrome (Antonius and Lipscomb 2001). The story with the scuticociliates is also complicated and members of the *Philaster* genus have a global reach like *Halofolliculina* (Sweet and Bythell 2012, 2015; Sweet et al. 2014; Sweet and Séré 2016). However, confusion arises when these ciliates are discussed as they were originally morphologically described as members of the genera *Porpostoma* or *Helicostoma* (Sweet and Bythell 2012; Sweet et al. 2012). The combination of metabarcoding and the construction of phylogenetic trees will help us to avoid this kind of confusion and to improve our knowledge on the ciliate diversity associated with corals.

Labyrinthulomycetes

Members of this group of ubiquitous and diverse unicellular stramenopiles are characterized by their production of an ectoplasmic network: a branched membrane network secreted through a unique organelle called the bothrosome (sagenogenetosome) that is involved in saprotrophic nutrient uptake (Bennett et al. 2017). Labyrinthulomycetes are found in diverse habitats, including marine and freshwater, and from the euphotic zone to the deep-sea. They have also been isolated from various substrates, including but not limited to algae, mangrove leaves, seagrass, mollusks and of course coral (Pan et al. 2017). The production of high levels of omega-3 polyunsaturated fatty acids, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), has made Labyrinthulomycetes commercially valuable (Carmona et al. 2003; Lee Chang et al. 2012). In some cases, labyrinthulids have been described as parasites, specifically in clams, nudibranchs and seagrass (McLean and Porter 1982; Ragone Calvo et al. 1998; Ragan et al. 2000; Muehlstein et al. 2018). Five Labyrinthulomycetes species have been isolated from corals (Raghukumar and Raghukumar 1991; Ben-Dov et al. 2009; Siboni et al. 2010), and diverse members have also been reported to be widely associated with the coral *Fungia sp.*, but the nature of their interactions has not been elucidated (Kramarsky-Winter et al. 2006; Ben-Dov et al. 2009; Siboni et al. 2010). Additionally, there has been a report proposing that labyrinthulids play a pathogenic role in the multifocal purple spot disease (MFPS) that affects

Caribbean sea fans. However, their causal role is doubtful due to their presence in healthy tissues as well (Burge et al. 2012; Dennis et al. 2020). There is still a debate as to the differences between MFPS disease and the other known sea fan disease, aspergillosis, albeit recent MFPS disease histology shows numerous opportunistic organisms in association with MFPS disease (Dennis et al. 2020), suggesting a microbial differentiation throughout pathology compared to aspergillosis.

Syndiniales

The Syndiniales, also called Marine Alveolate (MALV) Groups I–V, are a diverse collection of parasitic dinoflagellates that are known to infect a vast diversity of marine organisms, including metazoans, radiolarians, ciliates, and even other dinoflagellates. The Syndiniales kill their host resulting in the release of free-living parasitic dinospores that can last up to three days in the environment (Guillou et al. 2008). The diversity of Syndiniales within coral reef environments has not been fully explored, but a few studies have noted their presence in reef plankton, sediments, and the coral themselves (Clerissi et al. 2018; Qiu et al. 2021). Clerissi and colleagues discovered that a subgroup of Syndiniales (Group I) were one of the four main microeukaryotic taxa found within the cauliflower coral, *Pocillopora damicornis*. Other Group I species include *Ichtyodinium*, an endoparasite of fish embryos, and *Duboscquella*, a parasite of ciliates (Harada et al. 2007). Given their huge diversity in marine environments and their cosmopolitan range of known host organisms, Syndiniales represent a major lineage in need of additional exploration in reef environments, and in particular more data linking Syndiniales known only from environmental sequencing to their hosts to see which members of the reef environment are infected. The widespread parasitism of this clade, along with their accompanying ability to exert top-down control on host populations, suggests an important ecological role in coral holobionts.

Other dinoflagellates

Outside of the Syndiniales and Symbiodiniaceae, there are other dinoflagellates with potential roles in the coral holobiont. *Alexandrium spp.*, *Gymnodinium catenatum*, and *Prorocentrum lima* have all been found in coral samples with signs of black band disease (BBD). Furthermore, *Alexandrium* and *Gymnodinium* are known to produce paralytic shellfish toxins (Hold et al. 2001; Green et al. 2004; Sala et al. 2005). While not described as etiological agents in BBD, the disease is characterized as being caused by a microbial mat consortium and it may well be influenced by these dinoflagellates presence (Sekar et al. 2008). There is also indirect evidence that dinoflagellates may play a role in Stony Coral

Tissue Loss Disease—a coral disease rampaging through the Caribbean reefs. Indeed one of the bacteria named as a possible causal agent is known to be isolated from the ichthyopathogenic dinoflagellate *Cochlodinium polykrikoides* (Kim et al. 1999). Further evidence of the role of algae in coral disease stems from early reports back in 1985 where blooms of *Margalefidinium polykrikoides* and *Gonyaulax monilata* in Panama caused 100% mortality of surveyed corals (López-Cortés et al. 2019). However, some dinoflagellates do certainly appear to offer benefits to the host like their famous Symbiodiniaceae cousins. Taxa from the genus *Gyrodinium* for example have been recovered from corals and these species are known to produce mycosporine-like amino acids (MAAs). MAAs function as a UV-B absorbing compound (Klisch et al. 2001) and may therefore assist corals by reducing damage from UV (Gleason 1993). The genus *Blastodinium* has also been reported in corals (Sebens et al. 1996). Members of this genus are well known for parasitizing copepods for example (Skovgaard et al. 2012). We do not know if they play a similar role in the coral host or if they are only observed because the coral feeds on parasitized copepods. Similarly, *Amphidinium sp.* is another member from the family Gymnodiniaceae that has been recovered from corals; however, this taxa is also commonly observed as part of the coral diet (Sorokin 1973).

Molecular approaches to unveil the coral eukaryome

The majority of studies focused on coral-associated microeukaryote diversity have centered around disease dynamics with researchers isolating and identify associated microbes via microscopy. This low-throughput approach of microscopy and culturing is by necessity targeted and, as a result, relatively narrow in scope. It was not until the use of non-targeted, culture-independent approaches such as high-throughput metabarcoding (Taberlet et al. 2012) that we could retrieve from an environment not only what we might be looking for, but also everything else we did not even know was there. However, the vast majority of metabarcoding studies remain exclusively focused on prokaryotes. Some examine fungi (Cui et al. 2013), but few address the microeukaryotic community as a whole (Keeling and del Campo 2017). Indeed, thousands of publications have revealed a great diversity of bacteria associated with an almost equally diverse range of hosts (McFall-Ngai et al. 2013). This includes corals, where prokaryotic diversity has been extensively sampled and analyzed (Pollock et al. 2018), but few such studies have focused on microeukaryotes (Ainsworth et al. 2017). The paucity of DNA-based studies of coral-associated microeukaryotes reflects not only research biases, but also a serious technical issue that arises because

symbiotic microeukaryotes are evolutionarily related to their animal hosts, both being eukaryotes (del Campo et al. 2019). Among the barcoding genes used to infer the identity of microorganisms, the most widely used is the Small Subunit ribosomal RNA (SSU rRNA) gene, which is amplified by polymerase chain reaction (PCR) using primers that target the barcode gene. These approaches have been widely utilized to define phylogenetic diversity of free-living microeukaryotes for over 20 yrs (López-García et al. 2001; Moon-van der Staay et al. 2001), but their application to analyzing microeukaryotic components of communities associated with eukaryotic hosts (like animals) is challenging because host-derived sequences dominate the data (Parfrey et al. 2014; Wampach et al. 2017; Wilcox and Hollocher 2018). One solution to this is to develop PCR primers that amplify the SSU rRNA from wide range of eukaryotes, but exclude all or most metazoans. One such primer set developed to investigate parasites was recently shown to work on diverse microeukaryotes associated with diverse animal hosts (del Campo et al. 2019). More recently, improved versions of these primers have been released that will reduce the primer bias within certain eukaryotic groups and will increase the reported microeukaryotic diversity in microbiome samples (Bass and del Campo 2020; Minardi et al. 2021). Other recent studies have used alternative methods to explore the eukaryome, such as blocking primers (Clerissi et al. 2018). Both strategies have revealed novel microeukaryotes in the holobiont. Furthermore, metagenome assembled genomes can also be used and cases have already provided insights into the functional characteristics of this newly characterized diversity (Kwong et al. 2019).

The microeukaryotic component of the coral microbiome is the last major element that needs to be characterized in order to have a full picture of the holobiont. Metabarcoding and metagenomics, as well as promising methodologies to study the coral holobiont such as single-cell transcriptomics (Hu et al. 2020; Levy et al. 2021), will all play a crucial role in revealing the coral holobiont microeukaryotic diversity and function. We will likely see how the image we have of protist and fungi will move from being the usual suspects in disease to common members of the healthy holobiont. As part of the holobiont, microeukaryotes may also have an impact on the dynamics of other members of the coral microbiome. So, in time, they could potentially be utilized as a tool to assist corals through a changing climate in years to come (Reshef et al. 2006; Peixoto et al. 2021).

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Declarations

Conflict of interest The authors declare no competing interests to report.

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References

- Ainsworth TD, Fordyce AJ, Camp EF (2017) The Other Microeukaryotes of the Coral Reef Microbiome. *Trends Microbiol* 25:980–991
- Alker AP, Smith GW, Kim K (2001) Characterization of *Aspergillus sydowii* (Thom et Church), a fungal pathogen of Caribbean sea fans. *Hydrobiology fungal pathogen of Caribbean sea fan corals*. *Hydrobiologia* 460:105–111
- Amend AS, Barshis DJ, Oliver TA (2012) Coral-associated marine fungi form novel lineages and heterogeneous assemblages. *ISME J* 6:1291–1301
- Anthony S, Page C, Bourne D, Willis B (2008) Newly characterized distinct phases of the coral disease 'atramentous necrosis' on the Great Barrier Reef. *Dis Aquat Organ* 81:255–259
- Antonius A (1999) Halofolliculina corallasia, a new coral-killing ciliate on Indo-Pacific reefs. *Coral Reefs* 18:300
- Antonius A, Lipscomb D (2001) First protozoan coral-killer identified in the Indo-Pacific. *Atoll Res Bull* 493:1–21
- Bak RPM, Laane R (1987) Annual black bands in skeletons of reef corals (Scleractinia). *Mar Ecol Prog Ser* 38:169–175
- Ballesteros E (2006) Mediterranean coralligenous assemblages: A synthesis of present knowledge. *Oceanogr Mar Biol* 44:123–195
- Bass D, del Campo J (2020) Microeukaryotes in animal and plant microbiomes: Ecologies of disease? *Eur J Protistol* 76:125719
- Ben-Dov E, Kramarsky-Winter E, Kushmaro A (2009) An in situ method for cultivating microorganisms using a double encapsulation technique. *FEMS Microbiol Ecol* 68:363–371
- Bennett RM, Honda D, Beakes GW, Thines M (2017) Labyrinthulomycota. *Handbook of the Protists*. Springer, Cham, pp 507–542
- Bonthond G, Merselis DG, Dougan KE, Graff T, Todd W, Fourqurean JW, Rodriguez-Lanetty M (2018) Inter-domain microbial diversity within the coral holobiont *Siderastrea siderea* from two depth habitats. *PeerJ* 6:e4323
- Bourne DG, Boyett HV, Henderson ME, Muirhead A, Willis BL (2008) Identification of a ciliate (Oligohymenophorea: Scuticociliatia) associated with brown band disease on corals of the great barrier reef. *Appl Environ Microbiol* 74:883–888
- Brener-Raffalli K, Clerissi C, Vidal-Dupiol J, Adjeroud M, Bonhomme F, Pralong M, Aurelle D, Toulza E (2018) Thermal regime and host clade, rather than geography, drive Symbiodinium and bacterial assemblages in the scleractinian coral *Pocillopora damicornis* sensu lato. *Microbiome* 6:39

- Burge CA, Douglas N, Conti-Jerpe I, Weil E, Roberts S, Friedman CS, Harvell CD (2012) Friend or foe: The association of Labyrinthomyxetes with the Caribbean sea fan *Gorgonia ventalina*. *Dis Aquat Organ* 101:1–12
- Carmona ML, Naganuma T, Yamaoka Y (2003) Identification by HPLC-MS of Carotenoids of the *Thraustochytrium* CHN-1 Strain Isolated from the Seto Inland Sea. *Biosci Biotechnol Biochem* 67:884–888
- Clerissi C, Brunet S, Vidal-Dupiol J, Adjeroud M, Lepage P, Guillou L, Escoubas J-M, Toulza E (2018) Protists within corals: the hidden diversity. *Front Microbiol* 9:2043
- Craggs J, Guest J, Bulling M, Sweet M (2019) Ex situ co culturing of the sea urchin, *Mespilia globulus* and the coral *Acropora millepora* enhances early post-settlement survivorship. *Sci Rep* 9:12984
- Cróquer A, Bastidas C, Lipscomb D (2006a) Folliculinid ciliates: A new threat to Caribbean corals? *Dis Aquat Organ* 69:75–78
- Cróquer A, Bastidas C, Lipscomb D, Rodríguez-Martínez RE, Jordan-Dahlgren E, Guzman HM (2006b) First report of folliculinid ciliates affecting Caribbean scleractinian corals. *Coral Reefs* 25:187–191
- Cui L, Morris A, Ghedin E (2013) The human mycobiome in health and disease. *Genome Med* 5:63
- del Campo J, Pombert J-F, Šlapeta J, Larkum AWD, Keeling PJ (2017) The ‘other’ coral symbiont: *Ostreobium* diversity and distribution. *ISME J* 11:296–299
- del Campo J, Pons MJ, Herranz M, Wakeman KC, del Valle J, Vermeij MJA, Leander BS, Keeling PJ (2019) Validation of a universal set of primers to study animal-associated microeukaryotic communities. *Environ Microbiol* 21:3855–3861
- del Campo J, Bass D, Keeling PJ (2020) The eukaryome: Diversity and role of microeukaryotic organisms associated with animal hosts. *Funct Ecol* 34:2045–2054
- Dennis MM, Becker AAMJ, Freeman MA (2020) Pathology of multifocal purple spots, a nonspecific lesion morphology of Caribbean sea fans *Gorgonia* spp. *Dis Aquat Organ* 141:79–89
- Fine M, Loya Y (2002) Endolithic algae: an alternative source of photoassimilates during coral bleaching. *Proc B* 269:1205–1210
- Foissner W, Chao A, Katz LA (2008) Diversity and geographic distribution of ciliates (Protista: Ciliophora). *Biodivers Conserv* 17:345–363
- Galindo-Martínez CT, Weber M, Avila-Magaña V, Enríquez S, Kitano H, Medina M, Iglesias-Prieto R (2022). The role of the endolithic alga *Ostreobium* spp. during coral bleaching recovery. *Sci Rep* 12:2977
- Gifford DJ (1991) The Protozoan-Metazoan Trophic Link In Pelagic Ecosystems. *J Protozool* 38:81–86
- Gleason DF (1993) Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral *Porites astreoides*. *Limnol Oceanogr* 38:1452–1463
- Góes-Neto A, Marcelino VR, Verbruggen H, da Silva FF, Badotti F (2020) Biodiversity of endolithic fungi in coral skeletons and other reef substrates revealed with 18S rDNA metabarcoding. *Coral Reefs* 39:229–238
- Goldberg WM, Makemson JC, Colley SB (1984) *Entocladia endozoa* sp. nov., a pathogenic Chlorophyte: structure, life history, physiology, and effect on its coral host. *Biol Bull* 166:368–383
- Golubic S, Radtke G, Le Campion-Alsumard T (2005) Endolithic fungi in marine ecosystems. *Trends Microbiol* 13:229–235
- Green DH, Llewellyn LE, Negri AP, Blackburn SI, Bolch CJS (2004) Phylogenetic and functional diversity of the cultivable bacterial community associated with the paralytic shellfish poisoning dinoflagellate *Gymnodinium catenatum*. *FEMS Microbiol Ecol* 47:345–357
- Grossart H-P, Rojas-Jimenez K (2016) Aquatic fungi: targeting the forgotten in microbial ecology. *Curr Opin Microbiol* 31:140–145
- Guillou L, Viprey M, Chambouvet A, Welsh RM, Kirkham AR, Massana R, Scanlan DJ, Worden AZ (2008) Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). *Environ Microbiol* 10:3349–3365
- Harada A, Ohtsuka S, Horiguchi T (2007) Species of the Parasitic Genus *Duboscquella* are Members of the Enigmatic Marine Alveolate Group I. *Protist* 158:337–347
- Harris DL, Rovere A, Casella E, Power H, Canavesio R, Collin A, Pomeroy A, Webster JM, Parravicini V (2018) Coral reef structural complexity provides important coastal protection from waves under rising sea levels. *Sci Adv* 4:eaa04350
- Heyward AJ, Negri AP (1999) Natural inducers for coral larval metamorphosis. *Coral Reefs* 18:273–279
- Hold GL, Smith EA, Birkbeck HT, Gallacher S (2001) Comparison of paralytic shellfish toxin (PST) production by the dinoflagellates *Alexandrium lusitanicum* NEPCC 253 and *Alexandrium tamarense* NEPCC 407 in the presence and absence of bacteria. *FEMS Microbiol Ecol* 36:223–234
- Hu M, Zheng X, Fan C-M, Zheng Y (2020) Lineage dynamics of the endosymbiotic cell type in the soft coral *Xenia*. *Nature* 582:534–538
- Iha C, Dougan KE, Varela JA, Avila V, Jackson CJ, Bogaert KA, Chen Y, Judd LM, Wick R, Holt KE, Pasella MM, Ricci F, Repetti SI, Medina M, Marcelino VR, Chan CX, Verbruggen H (2021) Genomic adaptations to an endolithic lifestyle in the coral-associated alga *Ostreobium*. *Curr Biol* 31:1393–1402
- Janouškovec J, Horak A, Obornik M, Lukes J, Keeling PJ (2010) A common red algal origin of the apicomplexan, dinoflagellate, and heterokont plastids. *Proc Natl Acad Sci* 107:10949–10954
- Janouškovec J, Horák A, Barott KL, Rohwer FL, Keeling PJ (2012) Global analysis of plastid diversity reveals apicomplexan-related lineages in coral reefs. *Curr Biol* 22:R518–R519
- Janouškovec J, Horák A, Barott KL, Rohwer FL, Keeling PJ (2013) Environmental distribution of coral-associated relatives of apicomplexan parasites. *ISME J* 7:444–447
- Janouškovec J, Tikhonenkov DV, Burki F, Howe AT, Kolísko M, Mylnikov AP, Keeling PJ (2015) Factors mediating plastid dependency and the origins of parasitism in apicomplexans and their close relatives. *Proc Natl Acad Sci* 112:10200–10207
- Katz SM, Pollock FJ, Bourne DG, Willis BL (2014) Crown-of-thorns starfish predation and physical injuries promote brown band disease on corals. *Coral Reefs* 33:705–716
- Keeling PJ, del Campo J (2017) Marine Protists Are Not Just Big Bacteria. *Curr Biol* 27:R541–R549
- Kendrick B, Risk MJ, Michaelides J, Bergman K (1982) Amphibious microborers: bioeroding fungi isolated from live corals (Caribbean, South Pacific). *Bull Mar Sci* 32:862–867
- Kim CS, Lee SG, Lee CK, Kim HG, Jung J (1999) Reactive oxygen species as causative agents in the ichthyotoxicity of the red tide dinoflagellate *Cochlodinium polykrikoides*. *J Plankton Res* 21:2105–2115
- Klisch M, Sinha RP, Richter PR, Häder DP (2001) Mycosporine-like amino acids (MAAs) protect against UV-B-induced damage in *Gyrodinium dorsum* kofoid. *J Plant Physiol* 158:1449–1454
- Kramarsky-Winter E, Harel M, Siboni N, Ben Dov E, Brickner I, Loya Y, Kushmaro A (2006) Identification of a protist-coral association and its possible ecological role. *Mar Ecol Prog Ser* 317:67–73
- Kwong WK, del Campo J, Mathur V, Vermeij MJA, Keeling PJ (2019) A widespread coral-infecting apicomplexan with chlorophyll biosynthesis genes. *Nature* 568:103–107
- Kwong WK, Irwin NAT, Mathur V, Na I, Okamoto N, Vermeij MJA, Keeling PJ (2021) Taxonomy of the Apicomplexan Symbionts of Coral, including *Corallicolida* ord. nov., Reassignment of the Genus *Gemmocystis*, and Description of New Species

- Corallicola aquarius gen. nov. sp. nov. and Anthozoaphila gnarlus gen. nov. sp. nov. *J Eukaryot Microbiol* 68:e12852
- LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. *Curr Biol* 28:2570–2580
- Le Campion-Alsumard T, Golubic S, Hutchings P (1995) Microbial endoliths in skeletons of live and dead corals: *Porites lobata* (Moorea, French Polynesia). *Mar Ecol Prog Ser* 117:149–157
- Lee Chang KJ, Dunstan GA, Abell G, Clementson L, Blackburn S, Nichols P, Koutoulis A (2012) Biodiscovery of new Australian thraustochytrids for production of biodiesel and long-chain omega-3 oils. *Appl Microbiol Biotechnol* 93:2215–2231
- Leliaert F, Smith DR, Moreau H, Herron MD, Verbruggen H, Delwiche CF, De Clerck O (2012) Phylogeny and Molecular Evolution of the Green Algae. *CRC Crit Rev Plant Sci* 31:1–46
- Levy S, Elek A, Grau-Bové X, Menéndez-Bravo S, Iglesias M, Tanay A, Mass T, Sebé-Pedrós A (2021) A stony coral cell atlas illuminates the molecular and cellular basis of coral symbiosis, calcification, and immunity. *Cell* 184:2973–2987
- Lewis LA, McCourt RM (2004) Green algae and the origin of land plants. *Am J Bot* 91:1535–1556
- López-Cortés DJ, Núñez Vázquez EJ, Dorantes-Aranda JJ, Band-Schmidt CJ, Hernández-Sandoval FE, Bustillos-Guzmán JJ, Leyva-Valencia I, Fernández-Herrera LJ (2019) The State of Knowledge of Harmful Algal Blooms of *Margalefidinium polykrikoides* (a.k.a. *Cochlodinium polykrikoides*) in Latin America. *Front Mar Sci* 6:463
- López-García P, Rodríguez-Valera F, Pedrós-Alió C, Moreira D (2001) Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. *Nature* 409:603–607
- Marcelino VR, Verbruggen H (2016) Multi-marker metabarcoding of coral skeletons reveals a rich microbiome and diverse evolutionary origins of endolithic algae. *Sci Rep* 6:31508
- Massé A, Domart-Coulon I, Golubic S, Duché D, Tribollet A (2018) Early skeletal colonization of the coral holobiont by the microborer *Ulvophyceae Ostreobium* sp. *Sci Rep* 8:2293
- Mathur V, del Campo J, Kolisko M, Keeling PJ (2018) Global diversity and distribution of close relatives of apicomplexan parasites. *Environ Microbiol* 20:2824–2833
- McFall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Lošo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N, Kjelleberg S, Knoll AH, Kremer N, Mazmanian SK, Metcalf JL, Nealson KH, Pierce NE, Rawls JF, Reid A, Ruby EG, Rumpho ME, Sanders JG, Tautz D, Wernegreen JJ, Had MG, Bosch TCG, Carey HV, Domazet-lo T, Douglas AE, Dubilier N, Eberl G, Fukami T, Gilbert SF, Hentschel U, King N (2013) Animals in a bacterial world, a new imperative for the life sciences. *Proc Natl Acad Sci* 110:3229–3236
- McLean N, Porter D (1982) The Yellow-Spot Disease of *Tritonia diomedea* Bergh, 1894 (Mollusca: Gastropoda: Nudibranchia): Encapsulation of the Thraustochytriaceous Parasite by Host Amoebocytes. *J Parasitol* 68:243
- Minardi D, Ryder D, del Campo J, Fonseca VG, Kerr R, Mortensen S, Pallavicini A, Bass D (2022) Improved high throughput protocol for targeting eukaryotic symbionts in metazoan and eDNA samples. *Mol Ecol Resour* 22:664–678
- Montano S, Maggioni D, Liguori G, Arrigoni R, Berumen ML, Seveso D, Galli P, Hoeksema BW (2020) Morpho-molecular traits of Indo-Pacific and Caribbean *Halofolliculina* ciliate infections. *Coral Reefs* 39:375–386
- Moon-van der Staay SY, De Wachter R, Vulot D (2001) Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. *Nature* 409:607–610
- Moore RBB, Oborník M, Janoušek J, Chrudimský T, Vancová M, Green DHH, Wright SWW, Davies NWW, Bolch CJSJS, Heimann K, Šlapeta J, Hoegh-Guldberg O, Logsdon JMM, Carter DAA (2008) A photosynthetic alveolate closely related to apicomplexan parasites. *Nature* 451:959–963
- Morrison-Gardiner S (2002) Dominant fungi from Australian coral reefs. *Fungal Divers* 9:105–121
- Morse DE, Hooker N, Morse ANC, Jensen RA (1988) Control of larval metamorphosis and recruitment in sympatric agariciid corals. *J Exp Mar Bio Ecol* 116:193–217
- Muehlstein LK, Porter D, Short FT (1991) *Labyrinthula Zosteræ* Sp. Nov., The Causative Agent of Wasting Disease of Eelgrass, *Zostera Marina*. *Mycologia* 83:180–191
- Oborník M, Modrý D, Lukeš M, Černotíková-Štříbrná E, Cihlár J, Tesařová M, Kotabová E, Vancová M, Prášil O, Lukeš J (2012) Morphology, Ultrastructure and Life Cycle of *Vitrella brassicaformis* n. sp., n. gen., a Novel Chromerid from the Great Barrier Reef. *Protist* 163:306–323
- Pan J, del Campo J, Keeling PJ (2017) Reference Tree and Environmental Sequence Diversity of *Labyrinthulomycetes*. *J Eukaryot Microbiol* 64:88–96
- Parfrey LW, Walters W, Lauber CL, Clemente JC, Berg-Lyons D, Teiling C, Kodira C, Mohiuddin M, Brunelle J, Driscoll M, Fierer N, Gilbert JA, Knight R (2014) Communities of microbial eukaryotes in the mammalian gut within the context of environmental eukaryotic diversity. *Front Microbiol* 5:1–13
- Paulino GVB, Félix CR, Landell MF (2020) Diversity of filamentous fungi associated with coral and sponges in coastal reefs of north-east Brazil. *J Basic Microbiol* 60:103–111
- Peixoto RS, Sweet MJ, Vilella HDMM, Cardoso P, Thomas T, Voolstra CR, Høj L, Bourne DG (2021) Coral Probiotics: Premise, Promise, Prospects. *Annu Rev Anim Biosci* 9:265–288
- Pica D, Tribollet A, Golubic S, Bo M, Di Camillo CV, Bavestrello G, Puce S (2016) Microborer organisms in living stylasterid corals (Cnidaria, Hydrozoa). *Mar Biol Res* 12:573–582
- Pollock FJ, McMinds R, Smith S, Bourne DG, Willis BL, Medina M, Thurber RV, Zaneveld JR (2018) Coral-associated bacteria demonstrate phyllosymbiosis and cophylogeny. *Nat Commun* 9:4921
- Poynton SL, Whitaker B, Heinrich A (2001) A novel trypanoplasma-like flagellate *Jarrellia atramenti* n. g., n. sp. (Kinetoplastida: Bodonidae) and ciliates from the blowhole of a stranded pygmy sperm whale *Kogia breviceps* (Physeteridae): morphology, life cycle and potential pathogenicity. *Dis Aquat Organ* 44:191–201
- Qiu D, Huang L, Zhuang Y, Zhong Y, Tan Y, Li X, Liu S, Huang H, Lin S (2021) Dinoflagellate-targeted PCR reveals highly abundant and diverse communities of parasitic dinoflagellates in and near Zhubi Reef, South China Sea. *Coral Reefs* 40:1931–1939
- Ragan M, MacCallum G, Murphy C, Cannone J, Gutell R, McGladdery S (2000) Protistan parasite QPX of hard-shell clam *Merccenaria mercenaria* is a member of *Labyrinthulomycota*. *Dis Aquat Organ* 42:185–190
- Raghukumar C, Raghukumar S (1991) Fungal Invasion of Massive Corals. *Mar Ecol* 12:251–260
- Ragone Calvo L, Walker J, Bureson E (1998) Prevalence and distribution of QPX, Quahog Parasite Unknown, in hard clams *Merccenaria mercenaria* in Virginia, USA. *Dis Aquat Organ* 33:209–219
- Randall CJ, Jordán-Garza AG, van Woesik R, Randall CJ, Jordán-Garza AG, van Woesik R (2015) Ciliates associated with signs of disease on two Caribbean corals. *Coral Reefs* 34:243–247
- Ravindran J, Raghukumar C, Raghukumar S (2001) Fungi in *Porites lutea*: association with healthy and diseased corals. *Dis Aquat Organ* 47:219–228
- Reaka-Kudla ML (1997) The Global Biodiversity of Coral Reefs: A Comparison with Rain Forests. *Biodivers II Underst Prot Our Biol Resour Joseph Henry/National Academy Press, Washington DC*, pp. 83–108

- Reshef L, Koren O, Loya Y, Zilber-Rosenberg I, Rosenberg E (2006) The Coral Probiotic Hypothesis. *Environ Microbiol* 12:2068–2073
- Richter DJ, Berney C, Strassert JFH, Poh YP, Herman EK, Muñoz-Gómez SA, Wideman JC, Burki F, de Vargas C (2022) EukProt: A database of genome-scale predicted proteins across the diversity of eukaryotes. *Peer Community Journal* 2:e56
- Roach TNF, Little M, Arts MGI, Huckeba J, Haas AF, George EE, Quinn RA, Cobián-Güemes AG, Naliboff DS, Silveira CB, Vermeij MJA, Kelly LW, Dorrestein PC, Rohwer F (2020) A multiomic analysis of in situ coral–turf algal interactions. *Proc Natl Acad Sci* 117:13588–13595
- Rodríguez S, Cróquer A, Guzmán HM, Bastidas C (2009) A mechanism of transmission and factors affecting coral susceptibility to *Halofolliculina* sp. infection. *Coral Reefs* 28:67–77
- Rohwer FL, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser* 243:1–10
- Sala MM, Balagué V, Pedrós-Alió C, Massana R, Felipe J, Arin L, Illoul H, Estrada M (2005) Phylogenetic and functional diversity of bacterioplankton during *Alexandrium* spp. blooms. *FEMS Microbiol Ecol* 54:257–267
- Schlichter D, Zscharnack B, Krisch H (1995) Transfer of Photoassimilates from Endolithic Algae to Coral Tissue. *Naturwissenschaften* 82:561–564
- Schoch CLL, Seifert KAA, Huhndorf S, Robert V, Spouge JLL, Lesquesne CA, a., Chen W, Bolchacova E, Voigt K, Crous PW, Miller ANN, Wingfield MJ, Aime MCC, An KDK-D, Bai FYF-YFY, Barreto RWW, Begerow D, Bergeron MJM-JMJ, Blackwell M, Boekhout T, Bogale M, Boonyuen N, Burgaz ARR, Buyck B, Cai L, Cai Q, Cardinali G, Chaverri P, Coppins BJJ, Crespo A, Cubas P, Cummings C, Damm U, Beer ZW de, Hoog GS de, Del-Prado R, Dentinger B, Diéguez-Urbeondo J, Divakar PKK, Douglas B, Dueñas M, Duong TAA, Eberhardt U, Edwards JEE, Elshahed MSS, Fliegerova K, Furtado M, García MA, Ge Z-WZW, Griffith GWW, Griffiths K, Groenewald JZZ, Groenewald M, Grube M, Gryzenhout M, Guo LDL-D, Hagen F, Hambleton S, Hamelin RCC, Hansen K, Harrold P, Heller G, Herrera C, Hirayama K, Hirooka Y, Ho HMH-M, Hoffmann K, Hofstetter V, Högnabba F, Hollingsworth PM, Hong SBS-B, Hosaka K, Houbraken J, Hughes K, Huhtinen S, Hyde KDD, James TY, Johnson EMM, Johnson JEE, Johnston PRR, Jones EBGB, Kelly LJJ, Kirk PMM, Knapp DGG, Kõljalg U, Kovács GM, Kurtzman CPP, Landvik S, Leavitt SDD, Liggenstoffer ASS, Liimatainen K, Lombard L, Luangsa-ard JJJ, Lumbsch HTT, Maganti H, Maharachchikumbura SSNSN, Martin MPP, May TWW, McTaggart ARR, Methven ASS, Meyer W, Moncalvo MJM-MJM, Mongkolsamrit S, Nagy LGG, Nilsson RHRH, Niskanen T, Nyilasi I, Okada G, Okane I, Olariaga I, Otte J, Papp T, Park D, Petkovits T, Pino-Bodas R, Quaadvlieg W, Raja HAA, Redecker D, Rintoul TLL, Ruibal C, Sarmiento-Ramírez JM, Schmitt I, Schüßler A, Shearer C, Sotome K, Stefani FOPOP, Stenroos S, Stielow B, Stockinger H, Suetrong S, Suh SOS-OSO, Sung GHG-H, Suzuki M, Tanaka K, Tedersoo L, Telleria MTT, Tretter E, Untereiner WAA, Urbina H, Vágvölgyi C, Vialle A, Vu TDD, Walther G, Wang QMQ-MQM, Wang Y, Weir BSS, Weiß M, White MMM, Xu J, Yahr R, Yang ZLL, Yurkov A, Zamora JCJ-C, Zhang N, Zhuang WYW-YWY, Schindler D, de Beer ZW, de Hoog GS, Dieguez-Urbeondo J, Duenas M, Garcia MA, Hognabba F, Koljalg U, Kovacs GM, Sarmiento-Ramirez JM, Schussler A, Vagvolgyi C, Weiss M, (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc Natl Acad Sci* 109:6241–6246
- Sebens KP, Vandersall KS, Savina LA, Graham KR (1996) Zooplankton capture by two scleractinian corals, *Madracis mirabilis* and *Montastrea cavernosa*, in a field enclosure. *Mar Biol* 127:303–317
- Sekar R, Kaczmarek LT, Richardson LL (2008) Microbial community composition of black band disease on the coral host *Siderastrea siderea* from three regions of the wider Caribbean. *Mar Ecol Prog Ser* 362:85–98
- Siboni N, Rasoulouniriana D, Ben-Dov E, Kramarsky-Winter E, Sivan A, Loya Y, Hoegh-Guldberg O, Kushmaro A (2010) Stramenopile microorganisms associated with the massive coral *Favia* sp. *J Eukaryot Microbiol* 57:236–244
- Skovgaard A, Karpov SA, Guillou L (2012) The parasitic dinoflagellates *Blastodinium* spp. Inhabiting the gut of marine, Planktonic copepods: Morphology, ecology, and unrecognized species diversity. *Front Microbiol* 3:1–22
- Šlapeta J, Linares MC (2013) Combined Amplicon Pyrosequencing Assays Reveal Presence of the Apicomplexan “type-N” (cf. *Gemmocystis cylindrus*) and *Chromera velia* on the Great Barrier Reef, Australia. *PLoS One* 8:e76095
- Smith GW, Ives LD, Nagelkerken IA, Ritchie KB (1996) Caribbean sea-fan mortalities. *Nature* 383:487–487
- Sniezek JH, Coats DW, Small EB (1995) *Kyaroikeus cetarius* N. G., N. Sp.: A Parasitic Ciliate from the Respiratory Tract of Odonticete Cetacea. *J Eukaryot Microbiol* 42:260–268
- Song J, Kitamura S, Oh M, Kang H, Lee J, Tanaka S, Jung S (2009) Pathogenicity of *Miamiensis avidus* (syn. *Philasterides dicentrarchi*), *Pseudocohnilembus persalinus*, *Pseudocohnilembus hargisi* and *Uronema marinum* (Ciliophora, Scuticociliatida). *Dis Aquat Organ* 83:133–143
- Sorokin YI (1973) ON THE FEEDING OF SOME SCLERACTINIAN CORALS WITH BACTERIA AND DISSOLVED ORGANIC MATTER. *Limnol Oceanogr* 18:380–386
- Sweet MJ, Bythell JC (2012) Ciliate and bacterial communities associated with White Syndrome and Brown Band Disease in reef-building corals. *Environ Microbiol* 14:2184–2199
- Sweet M, Bythell J (2015) White Syndrome in *Acropora muricata*: Nonspecific bacterial infection and ciliate histophagy. *Mol Ecol* 24:1150–1159
- Sweet MJ, Séré MG (2016) Ciliate communities consistently associated with coral diseases. *J Sea Res* 113:119–131
- Sweet MJ, Jones R, Bythell JC (2012) Coral diseases in aquaria and in nature. *J Mar Biol Assoc United Kingdom* 92:791–801
- Sweet MJ, Burn D, Cróquer A, Leary P (2013) Characterisation of the Bacterial and Fungal Communities Associated with Different Lesion Sizes of Dark Spot Syndrome Occurring in the Coral *Stephanocoenia intersepta*. *PLoS ONE* 8:1–9
- Sweet MJ, Croquer A, Bythell JC (2014) Experimental antibiotic treatment identifies potential pathogens of white band disease in the endangered Caribbean coral *Acropora cervicornis*. *Proc R Soc B Biol Sci* 281:20140094
- Sweet M, Burian A, Fifer J, Bulling M, Elliott D, Raymundo L (2019) Compositional homogeneity in the pathobiome of a new, slow-spreading coral disease. *Microbiome* 7:139
- Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH (2012) Environmental DNA. *Mol Ecol* 21:1789–1793
- Tandon K, Pasella MM, Iha C, Ricci F, Hu J, O’Kelly CJ, Medina M, Kühl M, Verbruggen H (2023) Every refuge has its price: *Ostreobium* as a model for understanding how algae can live in rock and stay in business. *Semi Cell Dev Biol* 134:27–36
- Toller WW, Rowan R, Knowlton N (2002) Genetic evidence for a protozoan (phylum Apicomplexa) associated with corals of the *Montastraea annularis* species complex. *Coral Reefs* 21:143–146
- Tribollet A, Pica D, Puce S, Radtke G, Campbell SE, Golubic S (2018) Eucendolithic *Conchoecelis* stage (Bangiales, Rhodophyta) in the skeletons of live *Stylasterid* reef corals. *Mar Biodivers* 48:1855–1862

- Troeger V, Sammarco P, Caruso J (2014) Aspergillosis in the common sea fan *Gorgonia ventalina*: isolation of waterborne hyphae and spores. *Dis Aquat Organ* 109:257–261
- Upton SJ, Peters EC (1986) A new and unusual species of coccidium (Apicomplexa: Agamococcidiorida) from Caribbean scleractinian corals. *J Invertebr Pathol* 47:184–193
- van Oppen MJH, Blackall LL (2019) Coral microbiome dynamics, functions and design in a changing world. *Nat Rev Microbiol* 17:557–567
- Verbruggen H, Tribollet A (2011) Boring algae. *Curr Biol* 21:R876–R877
- Vohsen SA, Anderson KE, Gade AM, Gruber-Vodicka HR, Dannenberg RP, Osman EO, Dubilier N, Fisher CR, Baums IB (2020) Deep-sea corals provide new insight into the ecology, evolution, and the role of plastids in widespread apicomplexan symbionts of anthozoans. *Microbiome* 8:1–15
- Wampach L, Heintz-Buschart A, Hogan A, Muller EEL, Narayanasamy S, Laczny CC, Hugerth LW, Bindl L, Bottu J, Andersson AF, de Beaufort C, Wilmes P (2017) Colonization and Succession within the Human Gut Microbiome by Archaea, Bacteria, and Microeukaryotes during the First Year of Life. *Front Microbiol* 8:1–21
- Webster NS, Smith LD, Heyward AJ, Watts JEM, Webb RI, Blackall LL, Negri AP (2004) Metamorphosis of a Scleractinian Coral in Response to Microbial Biofilms. *Appl Environ Microbiol* 70:1213–1221
- Wilcox JJS, Hollocher H (2018) Unprecedented Symbiont Eukaryote Diversity Is Governed by Internal Trophic Webs in a Wild Non-Human Primate. *Protist* 169:307–320
- Williams AD, Brown BE, Putschim L, Sweet MJ (2015) Age-related shifts in bacterial diversity in a reef coral. *PLoS ONE* 10:1–16
- Woo YH, Ansari H, Otto TD, Klinger CM, Kolisko M, Michálek J, Saxena A, Shanmugam D, Tayyrov A, Veluchamy A, Ali S, Bernal A, del Campo J, Cihlář J, Flegontov P, Gornik SG, Hajdušková E, Horák A, Janouškovec J, Katris NJ, Mast FD, Miranda-Saavedra D, Mourier T, Naeem R, Nair M, Panigrahi AK, Rawlings ND, Padron-Regalado E, Ramaprasad A, Samad N, Tomčala A, Wilkes J, Neafsey DE, Doerig C, Bowler C, Keeling PJ, Roos DS, Dacks JB, Templeton TJ, Waller RF, Lukeš J, Oborník M, Pain A (2015) Chromerid genomes reveal the evolutionary path from photosynthetic algae to obligate intracellular parasites. *Elife* 4:1–41
- Yoon HS, Müller KM, Sheath RG, Ott FD, Bhattacharya D (2006) Defining the major lineages of red algae (Rhodophyta). *J Phycol* 42:482–492
- Yoon HS, Nelson W, Lindstrom SC, Boo SM, Pueschel C, Qiu H, Bhattacharya D, Nelson W (2016) Rhodophyta. *Handbook of the Protists*. Springer International Publishing, Cham, pp 1–45

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