Symbiosis in the Ocean Microbiome

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Jonathan P. Zehr and David A. Caron

Abstract

Symbiotic interactions are widespread in Earth's ecosystems including the marine environment. "Living together" describes a spectrum of interactions ranging from predation and parasitism to the positive interactions of commensalism and mutualism most commonly associated with the term symbiosis. Many well-known symbioses in the marine environment involve associations between microbes and multicellular organisms such as corals but there are diverse microbe-microbe symbiotic interactions that have been described for decades if not centuries from microscopic observations. Microbe-microbe symbioses have been challenging to study in part because of their small size, our inability to establish and culture them in the laboratory, and the ineffectiveness or inappropriateness of the methods that have been used to study macroscopic species. However, technical advances in nucleic acid sequencing, bioinformatics, isotopic approaches, and imaging have begun to provide new insights into these diverse and abundant interactions. The application of culture-independent approaches has revealed that microbial interactions in the marine microbiome range from metabolite exchanges between free-living planktonic cells to epibiotic and intracellular endosymbiotic interactions that bridge the symbiosis – organelle transition. Here we provide a brief overview of symbiosis and then focus on two specific vignettes in the oceanic plankton—N_2-fixing and planktonic rhizarian symbioses—that illustrate

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J. P. Zehr (✉)
University of California Santa Cruz, Santa Cruz, CA, USA
e-mail: zehrj@ucsc.edu

D. A. Caron
University of Southern California, Los Angeles, CA, USA
e-mail: dcaron@usc.edu

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how cutting-edge approaches and methodologies are providing new insights into the establishment and functioning of these associations.

Keywords

Microbial associations · Mutualism · Nitrogen fixation · Parasitism · Rhizaria · Symbiotic cyanobacteria

13.1 Introduction

Symbioses, intimate interactions between two or more organisms are ubiquitous in the ocean and have been important drivers of the evolution of life on Earth. Early observations of macroscopic microbial associations such as the cyanobacterial-fungal interaction in lichens led to the definition of the term symbiosis (DeBary 1879; Frank 1877; Sapp 2004). At that time, debate focused on whether the observed relationships were parasitic or a more beneficial and mutual interaction (Sapp 2004). The term symbiosis is now used to define a broad spectrum of obligate or facultative associations that have beneficial, detrimental, or neutral effects on their partners. Pure neutralism where neither organism has an effect on the other may not exist except in concept, but commensalism, where one organism benefits while the other is unaffected, is common. Mutualism, often confused with the broader term symbiosis, refers to partner organisms that mutually benefit from their association. Many symbioses are assumed to be mutualistic, although demonstrating the benefits to all partners within an association is often difficult and sometimes a matter of perspective. Parasitism, and by the broadest definition of symbiosis, predation, are common forms of symbioses in which the interaction is beneficial to one partner but clearly detrimental to the other(s).

In the marine environment, symbioses involving multicellular organisms from fish to corals are found throughout ocean habitats and across all domains of life (Apprill 2020). These include diverse associations such as bioluminescent bacteria associated with light organs in fish and squid (McFall-Ngai 2014), chemosynthetic bacteria associated with hydrothermal vent annelids and molluscs (Childress and Fisher 1992), sulfide-oxidizing bacteria with bivalves and seagrasses (Heide et al. 2012), and photosynthetic dinoflagellates in corals (Rosset et al. 2020). Microbial symbioses have increasingly become the focus of diverse research efforts as powerful new tools provide the ability to better characterize these relationships (Egan et al. 2020).

These well-known associations are partnerships between animals and their associated microbes but symbioses involving only single-celled species also abound (Wernegreen 2017), and may involve Bacteria including cyanobacteria, Archaea, and protists (predominantly microscopic, single-celled, eukaryotic organisms, Archibald et al. (2017)). These relationships are diverse and common in many habitats and are presumed to be analogs of early evolutionary events of organelle acquisition in eukaryotes. They range from loose associations to endosymbioses that in some cases have progressed towards organelle acquisition (Keeling et al. 2015). The marine microbiome (restricted here to microbe-microbe interactions) is a broad concept that spans the microbial species and/or genomes inhabiting the diverse habitats of the sea, from the dilute waters of the open ocean, to the complex sediment environment, and the many invertebrates and vertebrates that inhabit pelagic and benthic environments. The oceanic water column habitat, the subject of this overview, is dominated by microorganisms and includes many known and other yet-to-be-discovered symbiotic interactions.

Early microbiological investigations of microbes relied on microscopy and culturing in order to observe cells and obtain them for experimentation. Through these studies, it was realized that many microorganisms were more easily cultured as assemblages, or enrichments, presumably due to metabolic reliance on one another, but it was difficult to determine the underlying mechanisms of symbiosis or identify and work with the uncultured partners. Characterization and understanding of the metabolic interactions taking place within such "microbial consortia" using traditional methods has proven challenging, and the concept is still rudimentary with respect to the principles underlying the vast array of symbiotic interactions that occur among co-occurring microorganisms. Advances in culture-independent techniques, global environmental gene surveys, and advanced isotope and visualization technologies have facilitated the study of marine microbial interactions (Decelle et al. 2020, 2021; LeKieffre et al. 2018; Lima-Mendez et al. 2015; Meyer and Weis 2012). This chapter focuses on recent advances in understanding the symbiotic relationships within several important marine microbiomes (associations between protists and bacteria, specifically cyanobacteria) of the water column of the open ocean, perhaps the largest habitat. The diversity of these associations is briefly summarized and the reader is pointed to excellent reviews on these topics. From there, this chapter delves into examples of symbioses involving a few specific groups of planktonic microbes (mutualistic N_2 fixation, Sect. 13.3 and planktonic rhizarian protists, Sect. 13.4), and how cutting-edge technologies and methodological approaches are currently being employed to understand the metabolic and physiological interactions between the partners of these associations.

13.2 Physical Relationships and the Breadth of Microbial Symbioses

Virtually all microbes in the ocean interact to some degree, physically or energetically. Microbial interactions involve diverse physical and metabolic/chemical associations and range from those between unattached (but chemically-interacting) organisms, to attached (episymbiotic) relationships, and finally when one or more species is contained within the other (endosymbiosis) (Fig. 13.1). Beyond predator-prey or virus/parasite-host interactions, many of these relationships are beneficial to one of the associates but inconsequential to the other. One or both of the partners can require symbiosis in an obligate relationship. Obligate dependencies tend to require close physical association, such as surface attachment or intracellular localization.

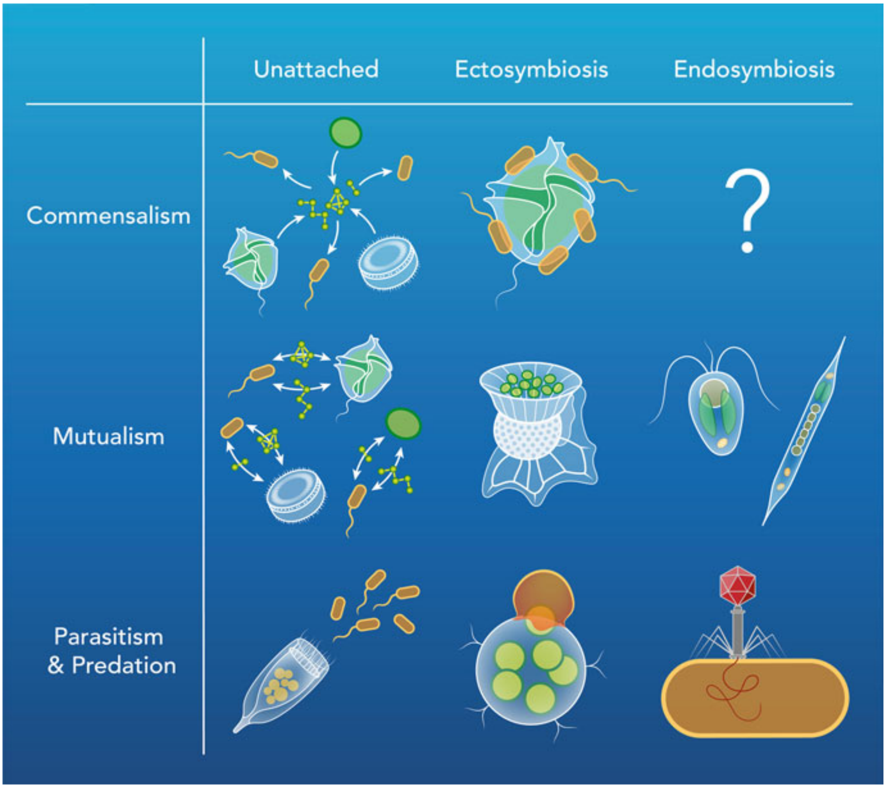

Fig. 13.1 Conceptual drawing of the diversity of symbiotic interactions ranging from commensalism to predation/parasitism (*y*-axis) and for physical relationships ranging from unattached but interacting partners to endosymbiotic situations (*x*-axis). Unattached commensalisms and mutualisms (left column) are typically established and mediated via chemical communication between the partners (note molecules and directional arrows), or interactions between free-living predators and prey. Ectosymbioses (middle column) involve the exchange of metabolites or nutrients between intimately associated partners (commensalism, mutualism) or predation/parasitism in which an attached parasite/predator extracts cells or cell contents from its prey (in the example shown, a vampyrellid amoeba is extracting algal cells from an algal colony). Endosymbioses (right column) are intimate physical relationships in which the host and symbionts share benefits (mutualism) or those in which one of the partners benefits to the detriment of the other (parasitism/predation). Examples of endosymbiotic commensalism are rare, or at least difficult to definitively confirm as true commensalisms

Microbial symbiotic interactions are generally based on diverse nutritional benefits (Braga 2016) and/or protection. Common nutritional benefits are acquired photosynthesis by otherwise heterotrophic hosts (Decelle et al. 2015; Norris 1996; Not et al. 2016; Stoecker et al. 2017), chemolithotrophic metabolisms of bacteria or archaea (Wrede et al. 2012), vitamin supplementation (Cruz-López et al. 2018), element acquisition (Zehr and Capone 2020), and general nutrition via the translocation of amino acids, carbohydrates, fatty acids, or other organic compounds (Balzano et al. 2015; LeKieffre et al. 2018; Liu et al. 2019).

13.2.1 Unattached Microbial Interactions

Open ocean microorganisms live in a dilute environment with respect to each other and nutrient molecules (Zehr et al. 2017). Cells are free-floating, swimming, or attached to other microorganisms or particles. Diffusion rapidly provides for exchanges of carbon compounds and nutrients among cells that are many cell diameters apart. Thus, generic metabolic dependencies exist between, for example, phytoplankton and bacteria that might exchange an organic carbon source for inorganic nutrients (Fig. 13.1, left column). Phytoplankton are generally a source of substrate for bacteria, and bacteria often provide specific nutritional advantages as sources of nutrients, vitamins, or trace elements (Cruz-López et al. 2018; Seymour et al. 2017; Yarimizu et al. 2018). At the microscale, boundary layers surrounding cells provide the opportunity for tighter metabolic interactions and most interactions happen at scales ≤ 100 μm (Cordero and Datta 2016; Stocker 2012). Microbial interactions between chemoheterotrophic bacteria and phytoplankton have been known for decades, starting with the concept of the phycosphere, which was suggested to be analogous to the rhizosphere in terrestrial ecosystems (Bell and Mitchell 1972; Cole 1982; Johansson et al. 2019; Seymour et al. 2017). There are many examples of loose physical associations of algae and bacteria whose interactions have played important roles over the course of evolution (Ramanan et al. 2016).

Algae growing in culture or in natural phytoplankton blooms have specific associated bacteria, although the nature of most interactions is poorly known (Buchan et al. 2014; Not et al. 2016; Sapp et al. 2007; Schäfer et al. 2002). It has long been assumed that microbial interactions were based on the exchange of metabolites. Recent work has identified a variety of specific metabolic interactions among planktonic microbes. Video microscopy has shown bacteria using chemotaxis to find lysing cells, which is important in particle-rich environments such as coastal waters (Smriga et al. 2016). Metabolic profiling of a diatom showed the effect of co-cultured bacteria on diatom metabolite profiles, although changes in growth rates were not detected (Paul et al. 2013). It has been shown that common oceanic phytoplankton can provide sulfur compounds to abundant free-living heterotrophic bacteria such as SAR11 (Durham et al. 2015). However, the metabolic interactions between microorganisms can be very specific. Through work with co-occurring bacteria in a diatom culture, Amin et al. (2015) demonstrated complex metabolic interactions that underlie positive effects on the growth of the individual microorganisms. Based on transcriptome analysis, *Sulfitobacter* sp. SA11 and *Phaeodactylum multiseries* grown together appeared to exchange carbon and nitrogen compounds for nutrition and possibly also for cell signaling and gene regulation. When grown together, the diatom had increased levels of transcripts for tryptophan synthesis and transport whereas *Sulfitobacter* appeared to increase transcript levels for indole acetic acid (IAA) production from tryptophan. IAA is not known to be utilized in bacteria but plays an important role in stimulating growth and cell division in plants and algae. The interactions are much more complex, however, since this alone could not explain the enhancement of growth. Other metabolic dependencies, such as the uptake of nitrate by *Sulfitobacter* and supply of ammonium to *P. multiseries* were suggested.

More complex and possibly evolutionarily important interactions have also been documented. Research on the biochemical interactions between choanoflagellates and some bacteria has revealed that certain bacteria are capable of producing compounds that elicit or inhibit changes between the solitary and colonial forms of these minute protists (Cantley et al. 2016; Woznica et al. 2016). Three distinct compounds produced by the bacterium *Algoriphagus machipongonensis* activate, enhance, or inhibit colony formation in the choanoflagellate *Salpingoeca rosetta* (Woznica et al. 2016). This remarkable responsiveness of the choanoflagellate life stage implies that chemically-mediated microbial interactions observed in symbioses may have influenced the development of coloniality in protists and by extension the evolution of multicellular species such as animals (Alegado et al. 2012; King et al. 2008).

There may be many metabolic dependencies among microbes in the open ocean: between free-floating, unattached cells, cells within the boundary layer, or attached to other cells (e.g., phytoplankton), or in close proximity to other cells on particles. The interactions at these spatial scales are complex, differ between different types of molecules, and are related to cell- and molecule sizes. Much is yet to be learned about the diversity of these types of interactions, which are at one end of the spectrum of symbiotic associations.

13.2.2 Ectosymbioses

The surfaces of organisms, including the intestinal linings of many animals (technically, still external to the animal), serve as substrata and often a source of nutrition for many types of microorganisms (Fig. 13.1, middle column; Fig. 13.2). Commensalism is perhaps most common among these associations, although that is unproven. All macroscopic and many microscopic organisms carry a myriad of microorganisms that may benefit from improved nutrition, improved environmental conditions, or protection from predators due to their associations with hosts but in turn cause no apparent harm or benefit to their hosts. These microbial associates, typically referred to as microbiomes, are not covered in detail in this review.

Numerous examples of species-specific bacterial taxa that colonize a variety of protists have been documented, although such symbioses are better characterized from non-marine ecosystems. For example, methanogenic Archaea are common ectosymbionts of ciliated protistan species in the rumen of cattle, benefiting from the production of hydrogen and formate produced by their hosts (Ushida 2018; Vogels et al. 1980). Another classic example is the colonization of some heterotrophic protists (protozoa) by ectosymbiotic bacteria in the hindgut of the termite,

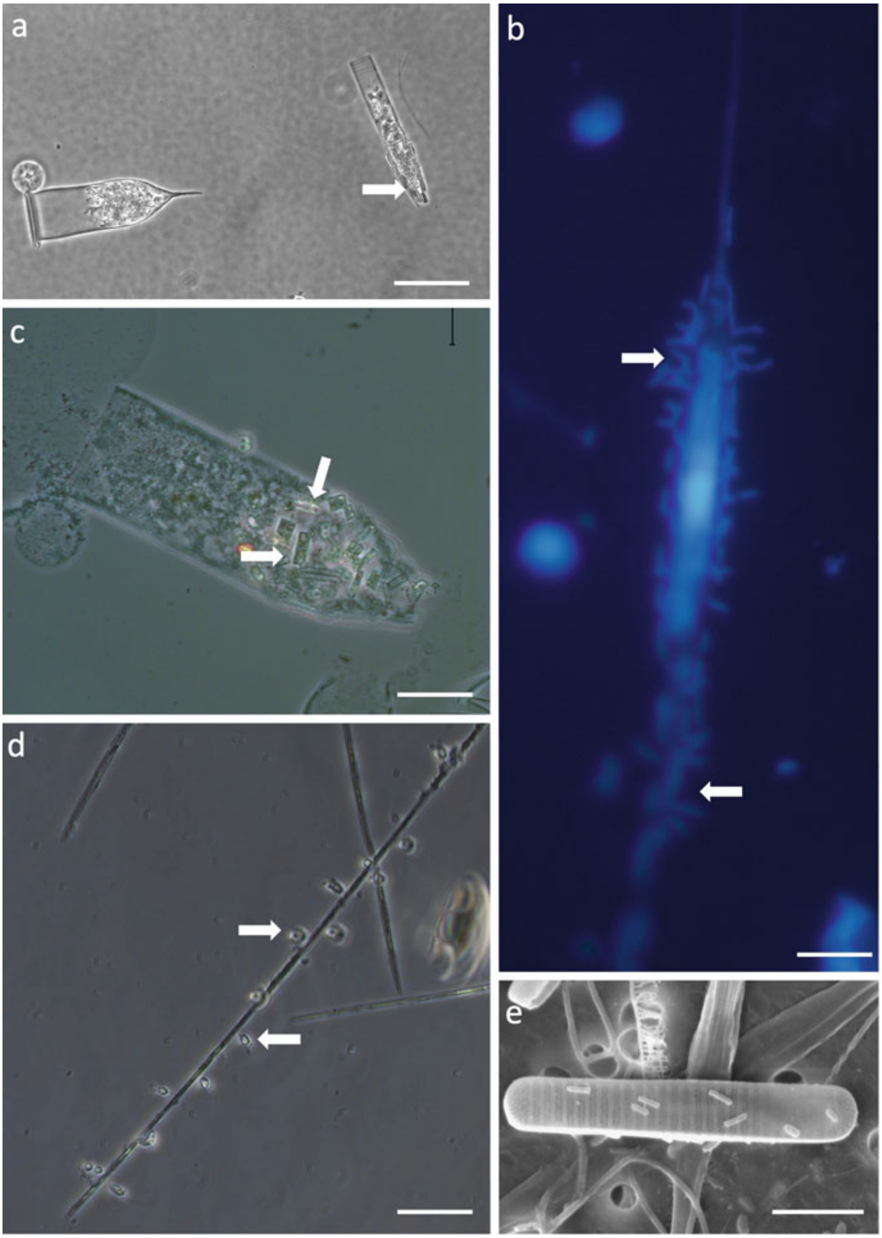

Fig. 13.2 Examples of ectocommensal relationships among microorganisms in the plankton. Algae are often found attached to protistan microzooplankton, or vice versa, including this tintinnid ciliate with multiple small diatoms attached to the outside of its lorica (arrows in **a**, **c**). The diatoms may obtain organic matter or nutrients released as waste by the host. A different tintinnid species in the same sample shows no colonization (**a**, specimen on left), implying specificity in the ectocommensalism on the right. An epifluorescence micrograph of a pennate diatom reveals many DAPI-stained bacteria (arrows) attached to its frustule (**b**). Minute heterotrophic flagellated protists (arrows in **d**) attached to a chain of thin diatoms feed on unattached suspended free-living

Cryptotermes cavifrons (Tamm 1982). The bacteria presumably benefit nutritionally from their association with the protists. It is unclear if the protists benefit directly from the presence of these ectosymbionts, although the host does obtain motility from the flagellar activity of the bacteria and may benefit somehow from the acquired motility. Similarly, ectosymbiotic bacteria instill magnetotactic behavior in some marine protists of anoxic sediments, a relationship that is thought to be mutualistic (Monteil et al. 2019).

Ectosymbiotic associations in marine systems are often non-specific involving random assortments of hosts and symbionts. A variety of small heterotrophic protists, for example, are commonly observed as ectosymbionts on larger photosynthetic protists (Taylor 1982). Diatoms are commonly observed with bacteria or various minute ciliated and flagellated protistan ectosymbionts attached to their siliceous frustules (Fig. 13.2b,d,e), or vice versa (Fig. 13.2a,c). The heterotrophic protists are typically bacterivorous and presumably benefit by feeding at somewhat higher bacterial abundances that characterize the phycosphere of diatoms or other microalgae (Seymour et al. 2017). Physical protection from suspension-feeding zooplankton that might consume small free-living protists may also be one of the benefits for the hitchhikers. While hosts and symbionts may constitute somewhat random pairings in most ectosymbioses, some relationships are remarkably species-specific. The bacterial ectosymbionts of the ciliated protist, *Zoothamnium niveum*, for example, are composed of a single bacterium that uniformly covers the exterior of the ciliate rather than a random assortment of bacterial types suggesting that the association itself is not random but a species-specific symbiotic interaction (Bauer-Nebelsick et al. 1996).

Some (perhaps most?) ectosymbiotic associations are comprised of a mixture of mutualistic, commensal, and even parasitic interactions. *Trichodesmium*, a colony-forming, free-living diazotrophic cyanobacterium, has long been known to have associated microorganisms including diverse proteobacteria, cyanobacteria, protists, and metazoa (Hewson et al. 2009; Sheridan et al. 2002; Siddiqui et al. 1992). An analysis of microbiomes of *Trichodesmium* colonies sampled from a number of stations in the Atlantic Ocean showed that there were diverse epibionts including alpha- and gamma Proteobacteria, that these assemblages differed from the surrounding water or particles, and that the same taxa were often present on colonies from numerous sampling sites (Frischkorn et al. 2017). The metabolic capabilities of the associates overlapped with *Trichodesmium* itself but extended the metabolic repertoire in the *Trichodesmium* aggregates suggesting that complex complementary metabolisms may contribute to *Trichodesmium*'s ecological success in the environment (Frischkorn et al. 2017; Gradoville et al. 2017). Similar findings of a complex yet commonly-occurring microbial community of *Trichodesmium* colonies have been reported by Lee et al. (2018) who concluded that the cyanobacterium's

Fig. 13.2 (continued) bacteria. Scanning electron micrograph of a pennate diatom shows several bacteria attached to its frustule (**e**). Marker bars are 50 (**a**), 10 (**b**, **d**, **e**) and 25 μm (**c**). Panel (**d**) courtesy of Richard Weinberg, University of Southern California ectosymbionts may play a role in colony-level nitrogen cycling. *Trichodesmium* also provides an example of “symbioses within symbioses.” Anderson observed a large amoeba associated with *Trichodesmium* colonies in the Sargasso Sea that harbored at least two different endosymbiotic bacteria (Anderson 1977). Such findings indicate that complex symbiotic associations are probably the rule rather than the exception.

Ectoparasites also exist although many of these are situations that may be transitory in nature, beginning with attachment to the exterior of the host and transitioning into an intracellular invasion. Colonization and ultimate invasion of diatoms by stramenopile (heterokont) flagellates in the genus *Pirsonia* (Kühn 1998; Kühn et al. 2004) and parasitic infections of diatoms and other phytoplankton by oomycete species are well-known examples of such cellular invasions (Garvetto et al. 2018; Hanic et al. 2009). A striking example involves the vampyrellid amoebae originally described from freshwater ecosystems but now identified from a variety of marine environments (Berney et al. 2013). These species attach to and excavate holes in the cell walls of some planktonic algae, and extricate chloroplasts from the algal cells or invade the cell to consume the contents from the inside (Hess 2017). These relationships muddy the lines between ecto- and endosymbiosis, parasitism, and predation, but they illustrate the potential for complexity and specificity of species interactions in the plankton.

Finally, the nature of some ectosymbioses (and other types of symbioses) may change with environmental situations. Ectocommensal oomycete protists may shift the relationship from a commensal interaction to a parasitic one if the host somehow becomes susceptible to attack. Many bacteria also appear to display this opportunistic ability, exhibiting algicidal activity under certain circumstances (Mayali and Azam 2004).

13.2.3 Endosymbioses

Perhaps the best known and most thoroughly studied symbioses are those that involve endosymbiosis. These are perhaps best known since they can be visualized. These interactions span mutualism to parasitism (Fig. 13.1, right column). Endosymbiosis greatly expands the capabilities of some macroorganisms with implications for adaptation and even evolution of new species (Kiers and West 2015) although how symbiosis has played a role is controversial (O’Malley 2015). Endosymbiosis with multicellular organisms takes on many forms and can involve complex cellular or organ development in the host. Single-celled symbiosis, the symbiosis between two unicellular (or sometimes colonial or filamentous) microorganisms provides simpler model systems for studying various stages of evolution of endosymbiosis and organelle evolution (Nowack and Melkonian 2010; Zehr 2015), although presenting methodological challenges because of their small size (Douglas and Raven 2003).

Intracellular symbiosis spans a spectrum of interactions from mutualistic relationships among partners to organelle acquisition where a symbiont has been subsumed into the cellular/genetic machinery of the host although few good model systems to study the transition from endosymbiosis to organelle exist (Douglas and Raven 2003). One well-known example is the amoeba *Paulinella* which harbors a cyanobacterial symbiont. The symbiont contains a highly reduced genome and extensive protein trafficking between host and symbiont has been demonstrated thereby exhibiting some characteristics of a plastid (Mackiewicz et al. 2012; Meheust et al. 2016; Nowack and Grossman 2012; Nowack and Melkonian 2010; Singer et al. 2017). The N_2-fixing cyanobacteria *Richelia*, UCYN-A, and the spheroid bodies of the freshwater diatom *Rhopalodia* (Trapp et al. 2012), are endosymbiotic and suggestive of evolution towards a N_2-fixing organelle, or “nitroplast” (Zehr 2015). Little is known about the biogeography, ecology, or biology of most of these symbioses.

There is a wide variety of presumedly mutualistic endosymbioses involving protistan hosts in the marine environment (Decelle et al. 2012; Gast and Caron 2001; Shaked and de Vargas 2006; Stoecker et al. 2017). The endosymbionts range from bacteria to eukaryotic algae and are found in a wide variety of heterotrophic protists. Photosynthetic endosymbionts of protists are particularly common and include dinoflagellates, haptophytes, prasinophytes, diatoms, and cyanobacteria. The potential benefit of “acquired photosynthetic ability” in otherwise heterotrophic protists is obvious to the host but less clear for the endosymbiont and has resulted in considerable research to understand how these associations are established and maintained and whether they constitute truly mutualistic relationships.

Parasitic and pathogenic interactions can be endosymbiotic and have evolutionary paths in common that are reflected in the pathogen and parasite symbiont genomes (Ochman and Moran 2001). While presumed to be common and important to the abundances of hosts in the ocean, the specific relationships and impacts of microbial pathogens on vulnerable hosts are still poorly known (Bratbak et al. 1996; Mayali and Azam 2004). Predatory microbes include diverse taxa that act in a variety of ways (Guerrero et al. 1986; Pasternak et al. 2013) including *Bdellovibrio*, which enter the periplasm of their hosts in order to replicate and lyse the prey (Sockett 2009). However, others exhibit a range of epibiotic or other life strategies that are evident in the proteomes (Pasternak et al. 2013). Predatory (Guerrero et al. 1986) or parasitic (Wang and Wu 2014) interactions have been suggested to be involved in the evolution of mitochondria.

Viruses are well-known obligate endosymbionts of bacteria, archaea, and eukaryotes and as such included in this category of pathogenic interactions. Much research has been performed during the past few decades to understand viral–host interactions in the ocean and those relationships have been the topic of many focused reviews (Breitbart 2012; Fuhrman and Suttle 1993; Tomaru et al. 2015; Weinbauer 2004).

Well-known protist-protist parasitic symbioses also exist in the plankton. Scholz et al. (2016) summarized the diverse phylogenetic groups of protists that function as parasites in many of these interactions. Knowledge of the extent of parasitic symbioses in the plankton has expanded rapidly in recent decades although the existence of these lifestyles among some protists has been known for over a century (noted in Coats (1999)). In particular, gene sequencing surveys of environmental

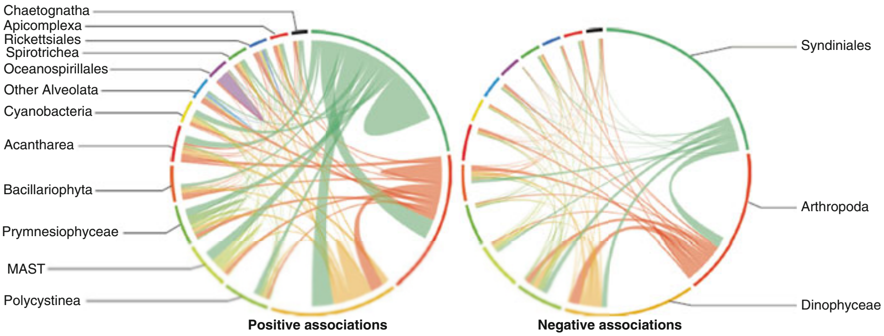

Fig. 13.3 A visualization (CIRCOS plot) showing interactions (positive and negative) between major bacterial, protistan, and animal taxonomic groups in surface waters from around the world ocean, as derived from the TARA Oceans dataset. Connectivity (ribbons between groups) may indicate a variety of types of interactions, including various symbiotic interactions (commensalism, mutualism, parasitism). Syndiniales (presumed parasites) constituted an important fraction of the interactions (Fig. 2A of Lima-Mendez et al. 2015; see for additional details)

samples have documented a much richer diversity and geographical distribution of protist-protist relationships than previously recognized. Early sequence surveys of this type revealed the presence of large numbers of genetic signatures of alveolate protists for which few if any cultured representatives existed (Moon-van der Staay et al. 2001). These sequences were most closely related to several dinoflagellate-like parasites that had been well-characterized from cultures and field studies (Coats and Park 2002). Gene sequencing studies since then have documented a considerable diversity and widespread occurrence of many previously unknown, dinoflagellate-like alveolates (the novel marine alveolates: MALV groups), provided refinements of their phylogeny and described relatives, suggested and applied approaches for identifying their hosts, and begun to characterize their ecological impacts in planktonic food webs (Groisillier et al. 2006; Guillou et al. 2008).

One benchmark study implicating the degree to which we may have massively underestimated the importance of protistan parasitic symbioses involving alveolates was provided by an analysis of the TARA Oceans expedition's genetic database of samples collected in surface waters throughout the world ocean (Lima-Mendez et al. 2015). A substantial fraction of the sequences examined in that study were identified as putative parasitic taxa, particularly marine alveolates among the Syndiniales (Fig. 13.3). That study employed network analysis and microscopy to begin to link the parasitic taxa to possible hosts, many of which apparently infect dinoflagellates (i.e., other alveolate taxa). An analysis of the "plankton protist interactome" also concluded that a substantial portion (18%) of all protist-protist interactions in nature may be parasitic, based on network analysis of information collected in a Protist Interaction DAtabase (Bjorbækmo et al. 2020).

Chytrids are microscopic, flagellated species within the Fungi that have been repeatedly observed as parasites of marine phytoplankton. A review by Kagami et al. (2007) summarized numerous reports of freshwater and marine host-chytrid parasitisms reported in the literature (see Table 1 in Kagami et al. 2007). Chytrids play important but still largely unquantified roles in altering phytoplankton community composition and in population demise. Conspicuous chytrid infections are particularly common during phytoplankton blooms, presumably a consequence of efficient transmission of the parasite at high prey abundance and perhaps coinciding with times when phytoplankton growth conditions are deteriorating.

Parasitic lifestyles also occur among several clades of stramenopile (heterokont) and cercozoan protists. Oomycetes within the stramenopiles ("water molds," not true Fungi) are common parasites that infect and kill a variety of diatom species (Garvetto et al. 2018; Hanic et al. 2009). Similarly, species of the genus *Pirsonia* are tiny heterotrophic flagellated protists that also infect and kill a variety of diatoms. The phylogeny of these protists has been confused in part due to their minute size and therefore limited morphological features. Based on sequence information, some species of *Pirsonia* appear to be Cercozoa while other species are closely related to stramenopile taxa. The genus continues to undergo phylogenetic revision (Kim et al. 2017; Kühn et al. 2004). *Cryothecomonas* is a cercozoan genus containing a few well-known predators/parasites. Large-scale infections of phytoplankton and algae occurring in sea ice have been reported for species of this genus (Stoecker et al. 1993; Tillmann et al. 1999).

13.3 Mutualistic Nutritional Symbioses: N_2 Fixation

An important and intriguing group of microbial mutualistic symbioses are based on nitrogen (N_2) fixation (Fig. 13.4). N_2-fixing symbioses are common and best known in terrestrial systems involving bacteria or cyanobacteria and multicellular plants (Davies-Barnard and Friedlingstein 2020; Elmerich 2007; Fisher and Newton 2002; Rai et al. 2003; Valentine et al. 2018). In marine systems, there are known N_2-fixing symbioses in virtually every habitat (Zehr and Capone 2020). N_2-fixing symbioses are found in a variety of benthic organisms (Fiore et al. 2010; Petersen et al. 2017) such as corals (Benavides et al. 2017; Davy et al. 2012) clams (Cardini et al. 2019) and shipworms (Carpenter and Culliney 1975; Distel et al. 1991). There are also associations with benthic macroalgae and plants that may be symbiotic (Capone 1983; Cardini et al. 2018; Head and Carpenter 1975). From a global perspective, some of the most important marine N_2-fixing symbioses are those involving planktonic protists and cyanobacteria (Foster and Zehr 2019; Thompson and Zehr 2013), which are responsible for a large fraction of the N supply fueling net community production and vertical export in well-lit, nutrient-poor surface waters (Böttjer et al. 2017; Karl et al. 1997; Zehr and Capone 2020), such as the open ocean gyres which we focus on here.

N_2 fixation is energetically expensive (requiring ATP and reductant) and the enzyme nitrogenase, which catalyzes the reduction of N_2 to NH_3, is extremely sensitive to oxygen inactivation (Fay 1992; Postgate 1998; Zehr and Capone 2020) and is found only in Bacteria and some Archaea. N_2-fixing symbiotic microorganisms, like their free-living counterparts, must deal with the challenges

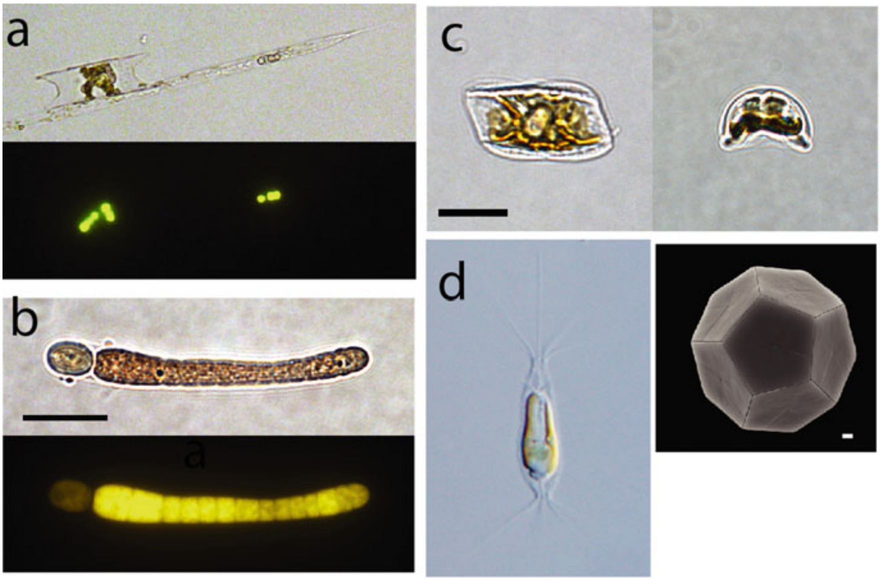

Fig. 13.4 Open ocean N_2-fixing cyanobacterial symbioses. a. Light and epifluorescence micrographs of the symbiosis between the diatom *Hemiaulus* and the heterocyst-forming cyanobacterium *Richelia* and *Rhizosolenia-Richelia* (**a**). Light and epifluorescence micrographs of *Calothrix*, a frequent epibiont on *Chaetoceros* diatoms (**b**). Scale bar 10 mm. Light micrographs of symbiotic marine rhopalodiacean diatoms *Epithemia catenata* sp. nov. (left) and *Epithemia pelagica* sp. nov. (right) (**c**). The cyanobacterial endosymbionts are visible as coccoid cells. Scale bar 10 μm (images courtesy of C. Schvarcz). Light micrograph of flagellated and calcified forms of the *Braarudosphaera bigelowii* (flagellated form previously identified as *Chrysochromulina*) symbiosis with UCYN-A cyanobacteria (*Candidatus* Atelocyanobacterium thalassa) (**d**). The flagellated form (left) courtesy of K. Hagino and Y. Takiano). Images in **a**, **b**, and calcified form of *B. bigelowii* in **d** from Zehr and Capone (2021), with permission

of obtaining energy and avoiding oxygen damage, either from photosynthetically produced oxygen, or oxygen in the environment. Cyanobacteria have evolved several strategies for avoiding oxygen inhibition of N_2 fixation by photosynthetically evolved oxygen, including temporal and spatial separation of photosynthetic and fixation processes (Berman-Frank et al. 2003; Fay 1992). Symbiotic cyanobacteria use variations of these strategies to avoid oxygen inactivation, including symbiosis with photosynthetic protists.

Photosymbiotic N_2-fixing symbioses are common and diverse in the open ocean. Common N_2-fixing symbionts involve cyanobacteria, ranging from heterocyst-forming filamentous to unicellular taxa (Fig. 13.4) with a wide variety of protistan algae including diatoms and haptophytes (Foster and Zehr 2019). These are different than most photosymbioses because sometimes both partners are photosynthetic. Single-celled N_2-fixing symbioses are of evolutionary interest since they are analogous to the symbiotic events leading to the evolution of organelles, in this case leading to a N_2-fixing organelle ("nitroplast") (Zehr 2015).

In contrast to photosymbioses involving cyanobacteria, N_2-fixing non-cyanobacterial diazotroph (NCD) symbioses (presumed Bacteria and Archaea that are chemoheterotrophic or photoheterotrophic) in marine protists are much less commonly known, or possibly just not yet discovered. There are reports of NCD nitrogenase (*nifH*) gene sequences associated with phytoplankton cells (Bombar et al. 2013; Farnelid et al. 2010). Farnelid et al. (2010) showed that bacterial *nifH* genes were associated with heterotrophic dinoflagellates that had previously been noted as bearing cyanobacteria associates (Lucas 1991). Analysis of TARA Oceans metagenomic data showed that *nifH*-containing bacteria occurred in large plankton size-classes and were either particle-associated or associated with larger organisms (Gradoville et al. 2017; Karlusich et al. 2020). However, such associations are scarce (Farnelid et al. 2020).

The longest-known marine N_2-fixing symbioses involve diatoms and heterocyst-forming cyanobacteria (Caputo et al. 2019; Foster and Zehr 2019; Heninbokel 1986; Villareal 1989, 1990, 1991; White et al. 2007). Three common associations with the centric chain-forming diatom genera *Rhizosolenia*, *Hemiaulus*, and *Chaetoceros* (Fig. 13.4) have been observed in the environment, sometimes in blooms (Carpenter et al. 1999; Villareal 1994; Villareal et al. 2011). The filamentous cyanobacteria form short chains of vegetative cells with a terminal heterocyst and are epibiotic attached to the external surface (*Calothrix rhizosoleniae*), or endosymbiotic within the diatom frustule (*Richelia intracellularis*) (Villareal 1990, 1992). *R. intracellularis* in *Rhizosolenia* is between the frustule and the cell membrane (Caputo et al. 2019; Pyle et al. 2020) whereas in *Hemiaulus hauckii* they may be inside the diatom cell membrane (Caputo et al. 2019).

The two cyanobacterial genera (*Richelia* and *Calothrix*) are morphologically similar (Fig. 13.4) but there is genetic diversity among the strains and they are genetically distinct suggesting host-specificity (Caputo et al. 2019; Foster and Zehr 2006; Janson et al. 1999). No permanent stable cultures of these associations exist but cultures have been maintained sufficiently long in order to facilitate physiological experiments on growth and nutrient uptake (Pyle et al. 2020; Villareal 1989, 1990). The epibiont *Calothrix* sp. SC01 has been maintained in stable culture without the diatom symbiont (Foster et al. 2010). There is likely to be more uncultured diversity in the environment (Hilton et al. 2015) and it seems likely that there are strains of the diatom genera that do not harbor symbionts (Pyle et al. 2020). The diatom symbionts have been observed throughout the oceans in imaging and -omic data from TARA Oceans (Karlusich et al. 2020). It is unclear whether observed free *Richelia*-like filaments are truly free-living or lost from host diatom cells. It has not been determined whether the cyanobacterial symbionts are vertically transferred from generation to generation during cell division, or horizontally transferred via a free-filament stage in the environment. They have been observed in *Trichodesmium* aggregates (Momper et al. 2015). It is also unclear how they might be regained through the diatom frustule during the horizontal transfer from the environment. There have been reports of unattached cyanobacteria identified as *Richelia* (or unassociated diatoms, see Caputo et al. (2019)), but it is not clear whether or not they are genetically the same as those in symbiosis nor is it known how they would penetrate the silica frustule to be obtained from the environment to reinfect the diatoms.

The functions of the diatom symbioses are not entirely understood. It is clear that N_2 is fixed by the cyanobacterial heterocyst and that the N is rapidly transferred throughout the diatom (Foster et al. 2011). Vegetative cells are photosynthetic and likely fuel N_2 fixation in the heterocyst possibly along with photosynthesis by the host. N_2 fixation rates have been measured in natural populations and cultures (Carpenter et al. 1999; Foster et al. 2011; Villareal 1990) and are sufficient to support growth rates on the order of 1 day^{-1} or even greater (Pyle et al. 2020). Metatranscriptomic analyses of natural populations suggest that cyclic phosphorylation might be important in *Richelia* associated with *Hemiaulus* and that a small antisense RNA may be involved in regulating cyclic photosynthesis by up- or down-regulating NADH dehydrogenase (Hilton et al. 2015).

Some genome reduction has been noted in the diatom symbionts compared to the epibiont *Calothrix* spp. SC01 (Hilton et al. 2013), perhaps an indication of movement towards organellogenesis. The genomic differences and presence or absence of genes coincide with physiological differences such as the response to nitrate (Pyle et al. 2020) and their intracellular location (Caputo et al. 2019). In nature, the growth of the symbionts needs to be coordinated with the growth of the host to maintain the partnership and metatranscriptomic studies show that there are coordinated rhythms of gene expression (Harke et al. 2019). However, in laboratory culture cell division of the diatom and cyanobacterium can become uncoupled leading to loss of the symbiont (Villareal 1989).

In freshwater, unicellular N_2-fixing cyanobacteria known as spheroid bodies are known to be associated with rhopalodian diatoms (Floener and Bothe 1980; Nakayama et al. 2011; Prechtl et al. 2004). These cyanobacteria are phylogenetically related to the coccoid cyanobacteria *Cyanothece* and *Crocosphaera* but have lost photosynthetic capability yet retained *nif* genes and fix N_2 (Bothe et al. 2010; Floener and Bothe 1980; Nakayama et al. 2014; Prechtl et al. 2004). The genome of the spheroid body of the diatom *Epithemia* has been sequenced and shows great genome reduction including loss of both photosystems and RuBisCO (Nakayama et al. 2014). Strains of a marine rhopalodian diatom have been isolated that also contain N_2-fixing spheroid bodies (Schvarcz et al. in press) (Fig. 13.4). These marine strains have likely been previously overlooked since the spheroid bodies lack pigments, but *nif* gene surveys show that they are widely distributed (Schvarcz et al. in press). They are similar in morphology and function to the UCYN-A symbionts discussed below.

A symbiosis between a unicellular cyanobacterium similar to the spheroid bodies in diatoms (now called UCYN-A or *Candidatus* Atelocyanobacterium thalassa) and a haptophyte (prymnesiophyte of the *Braarudosphaera bigelowii* group) was discovered in the last few decades (Hagino et al. 2013; Krupke et al. 2013, 2014; Thompson et al. 2012) following the initial report of a cyanobacterial *nifH* sequence from Station ALOHA in the North Pacific (Zehr et al. 1998). Similar to the spheroid bodies of freshwater *Rhopalodia*/*Epithemia*, the UCYN-A genome has been greatly reduced lacking even more enzymes than the *Epithemia* symbiont (Bothe et al. 2010;

Tripp et al. 2010). Tracer experiments demonstrated that N fixed by the symbiont was rapidly transferred to the haptophyte in exchange for fixed C (Thompson et al. 2012) and they fix N_2 in natural populations in a wide variety of locations including the Arctic (Cabello et al. 2016; Harding et al. 2018; Krupke et al. 2013, 2014; Martinez-Perez et al. 2016). The symbiotic relationship has led to physiological or genomic adaptations of the eukaryotic host such as the lack of ability to use exogenous nitrate (Mills et al. 2020). However, the full genome sequence of the haptophyte host has yet to be obtained. The UCYN-A symbioses exist as a cluster of closely related sublineages (termed UCYN-A1, UCYN-A2, etc.) that appear to have a specificity of host and symbiont (Cornejo-Castillo et al. 2019; Farnelid et al. 2016; Zehr et al. 2016).

The haptophyte host of one of the strains (the host of the strain UCYN-A2) is *Braarudosphaera bigelowii*, which has pentalith calcareous plates (Fig. 13.4) and has been found in sediments (Takayama 1972). Calcified cells collected in Japanese waters contain a spheroid body which was shown to be UCYN-A by nitrogenase (*nifH*) gene PCR (Hagino et al. 2013). The calcareous form appears to be only one life stage of the symbiotic cells in Japanese waters, as it also exists in a flagellated form with haptonema previously named *Chrysochromulina parkerae* (Hagino et al. 2013). Thus far in open ocean waters only the uncalcified forms have been reported by CARD-FISH observations (with the smaller UCYN-A1 form being the most abundant), but CARD-FISH procedures might destroy the calcareous plates.

Numerous other associations between cyanobacteria and protists have been observed microscopically that are possibly (but not proven) N_2-fixing symbioses (Foster and Zehr 2019). Among these are the diatom *Climacodium* and a unicellular cyanobacterium morphologically and phylogenetically similar to the *Crocosphaera*/*Cyanothece* group (Carpenter and Janson 2000). Cyanobacteria are associated with heterotrophic dinoflagellates, tintinnids, radiolarians, and amoebae (Foster and Zehr 2019). Some of such associations are suspected not to be N_2-fixing because the cyanobacterial cells are morphologically more similar to non-N_2-fixing cyanobacteria and are closely related to the non-N_2-fixing genera *Synechococcus* and *Prochlorococcus* (Foster et al. 2006a, b).

Knowledge of the UCYN-A N_2-fixing symbiosis came from targeted molecular (i.e., PCR-based) approaches. New technologies including metagenomic and metatranscriptomic approaches are providing new information on N_2-fixing symbioses. Data from the TARA Oceans dataset contained the complete sequence of the previously sequenced UCYN-A genome and was used to characterize UCYN-A gene expression (Cornejo-Castillo et al. 2016) as well as enabling application of FISH probes to characterize the distribution of UCYN-A symbioses across wide geographic areas (Cabello et al. 2016). Information on the host genome sequence is yet unpublished but metagenomic and metatranscriptomic studies are beginning to provide information on the host (Vorobev et al. 2020). The flagellated form of the symbiosis has been maintained in a nonaxenic culture finally facilitating biological experiments on symbiotic function.

13.4 Planktonic Rhizaria and Their Spectrum of Symbioses in the Ocean

The larger planktonic Rhizaria, specifically those species now encompassed by the clade Retaria containing the well-known Foraminifera and Radiolaria, are common and conspicuous protists that are found globally in the oceanic marine plankton. Planktonic Foraminifera contains a small number (roughly three dozen) of extant recognized species, whereas the Radiolaria are highly speciose. Radiolarian phylogeny has been the subject of considerable revision over the last few decades (Sierra et al. 2013), but the present-day Radiolaria includes the commonly encountered polycystine groups (Nassellaria, Spumellaria, Collodaria) and the Acantharia. Retaria includes some of the largest and most beautiful protistan taxa, with solitary adult specimens that can reach up to a centimeter or more in diameter and colonial forms that can form gelatinous ribbon-like structures exceeding one meter in length (Anderson 1983a; Hemleben et al. 1988).

Large planktonic Retaria have been a topic of fascination and biological research since the benchmark descriptive work and magnificent illustrations of Ernst Haeckel in the nineteenth century (Haeckel 1862, 1887). These organisms produce complex networks of pseudopodia, spider web-like extensions of their cellular cytoplasm that are used to entangle, capture, and digest prey and, in some species, harbor endosymbiotic algae (Fig. 13.5). All planktonic Foraminifera and Radiolaria are heterotrophic, feeding as generalists on a wide variety of planktonic organisms (Anderson 1983a, 1993; Hemleben et al. 1988; Swanberg 1983; Swanberg and Caron 1991). Additionally, many taxa within these groups form intricate skeletal structures of calcium carbonate (calcite) by the Foraminifera, silica (opal) in the nassellarian and spumellarian Radiolaria, or strontium sulfate (celestite) in the acantharian Radiolaria. The calcium carbonate and silica skeletons are fossilizable, making those specimens a mainstay of micropaleontological studies and paleoclimatological reconstructive work (Haq and Boersma 1998).

Pertinent to this chapter, planktonic Retaria take part in a variety of symbiotic interactions including commensalism, mutualism, and parasitism. Some species that participate in mutualistic associations harbor more or less intact photosymbiotic algae (an association that did not go unnoticed by Haeckel in the nineteenth century; Figs. 13.5 and 13.6), while others retain ‘reduced’ photosymbiotic algae or merely the chloroplasts of photosynthetic prey. As such, planktonic Retaria provide rich subject matter for research on the establishment and maintenance of symbiotic associations in the oceanic plankton, the physiological interactions that take place between hosts and symbionts, and the ecological consequences of those associations. However, the hosts are also extremely delicate creatures that have proven exceptionally difficult to culture in the laboratory although they can be hand-collected by SCUBA divers or gently collected in plankton nets and reared in the lab for days to a few weeks (Bé 1982; Kimoto 2015; Swanberg 1979). Beyond basic descriptions, simple experimental manipulations, and speculation regarding the nature of these associations, understanding of the roles of host and symbionts was limited until recently. This situation has begun to change due to the application of novel imaging

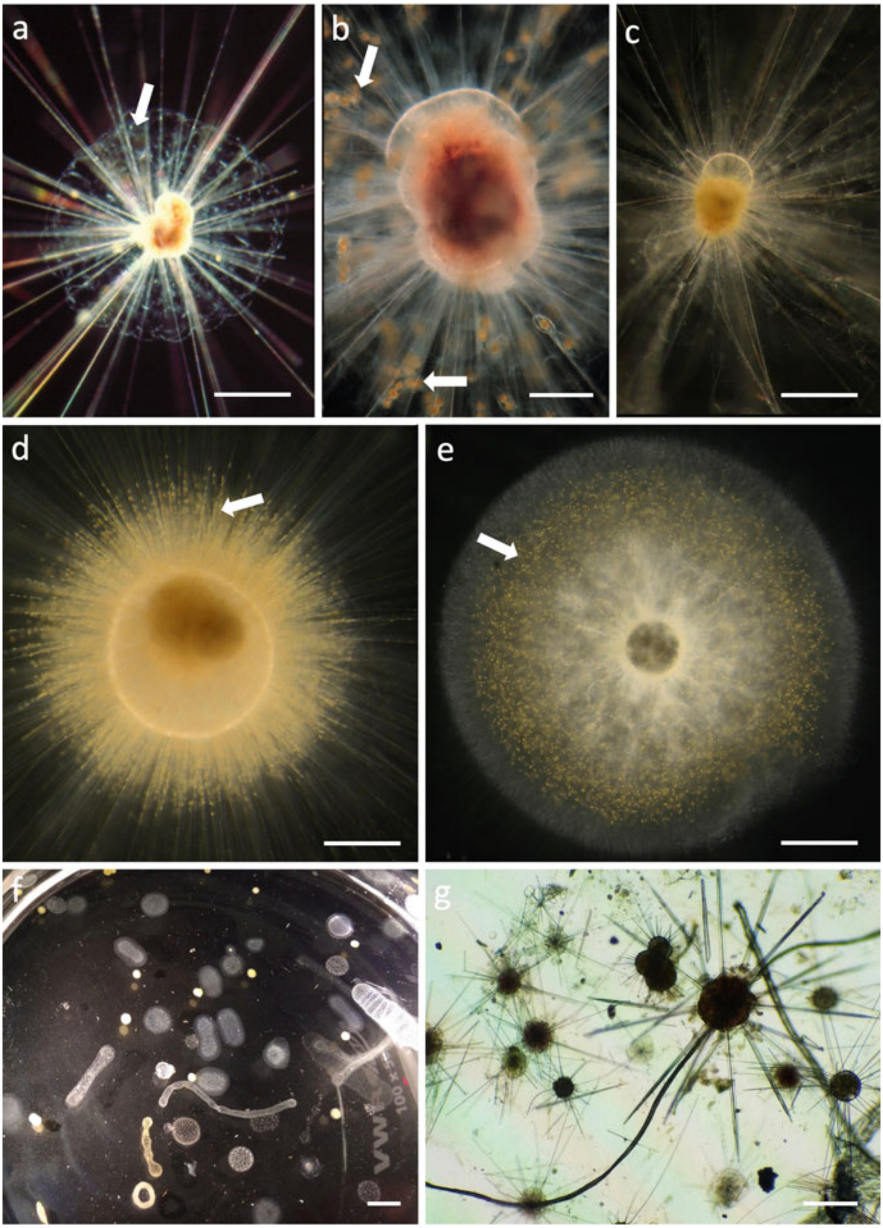

Fig. 13.5 Planktonic Retaria from the oligotrophic ocean. The planktonic foraminiferan, *Hastigerina pelagica* produces long spines from its calcite skeleton on which it drapes sticky pseudopodia for capturing prey (**a**), and a bubble capsule to aid flotation (arrow in **a**). This species is often observed with commensal photosynthetic dinoflagellates in its pseudopodial network (**b**, arrows). *Globigerinella* (*aquilateralis*) *siphonifera* possesses a dense pseudopodial network and harbors either tiny endosymbiotic haptophytes (**c**, visible as yellow-brown color) or prasinophyte symbionts. The planktonic foraminiferan, *Orbulina universa*, with several thousand dinoflagellate endosymbionts (*Pelagodinium béii*) visible in its pseudopodia (**d**). The solitary radiolarian, *Thalassicolla nucleata*, with thousands of dinoflagellate endosymbionts, *Brandtodinium nutricula*

methodologies and genetic approaches. The examples below illustrate the breadth of retarian symbioses and how novel approaches are providing new insights and understanding into the functioning of these important and widespread symbioses.

13.4.1 Commensalistic and Mutualistic Photosymbioses Among Planktonic Retaria

Planktonic Retaria are voracious and highly efficient at capturing a tremendous array of sizes and types of planktonic prey ranging from bacteria to small or weak-swimming metazoa that are incapable of escaping the extensive and sticky pseudopodial networks of these specimens (Anderson 1993; Swanberg and Caron 1991) (Fig. 13.5a,c–e). Nonetheless, a few photosynthetic protistan species often found enmeshed within the pseudopodia of some planktonic Foraminifera appear to be immune to capture and digestion. The most commonly observed of these associates are photosynthetic dinoflagellates within the genus *Pyrocystis* and a few other dinoflagellate genera within the pseudopodial networks of the planktonic foraminiferan, *Hastigerina pelagica*. *H. pelagica* otherwise harbors no algal symbionts (unlike numerous other planktonic foraminiferal species). Other ectosymbiotic algae also have been observed occasionally associated with Foraminifera (Decelle et al. 2015). The photosynthetic dinoflagellates appear to suffer no harm from their host nor do they appear to provide it any benefit, therefore these associations have generally been thought to be commensal in nature (Fig. 13.5a,b; also see Figs. 5.3 and 5.4 in Hemleben et al. 1988). Physical protection from predation, acquired buoyancy, and nutritional supplementation have been proposed as possible benefits to the commensal algae although none of them have been substantiated.

Far more common and more extensively studied are photosymbioses that occur between numerous species of planktonic Retaria and a variety of photosynthetic protists (Figs. 13.5c–e and 13.6). The photosynthetic endosymbionts that occur in these innately heterotrophic species of Retaria often number in the thousands within an individual host. The associations are established by juvenile specimens each generation (i.e., horizontal transmission) and include situations that are probably the closest to true mutualisms among protistan symbioses (Figs. 13.5 and 13.6). However, more recent studies are also revealing that the relationship between symbionts and hosts varies among the many described associations and actually span a broad range of interactions from mutualism to almost complete organelle acquisition, as detailed below.

◀

Fig. 13.5 (continued) (**e**). Darkfield image of the contents of a plankton tow (1 mm mesh, 1 m diameter net) from the North Pacific Subtropical Gyre placed in a crystallizing dish showing the dominance of a variety of colonial Radiolaria (**f**). Silhouette micrograph of a 200 μm mesh plankton tow from the same location showing the dominance of Acantharia (one multi-chambered foraminiferan is also visible). Marker bars are 500 (**a**, **c**), 200 (**b**, **d**, **e**), 1000 (**f**) and 100 μm (**g**)

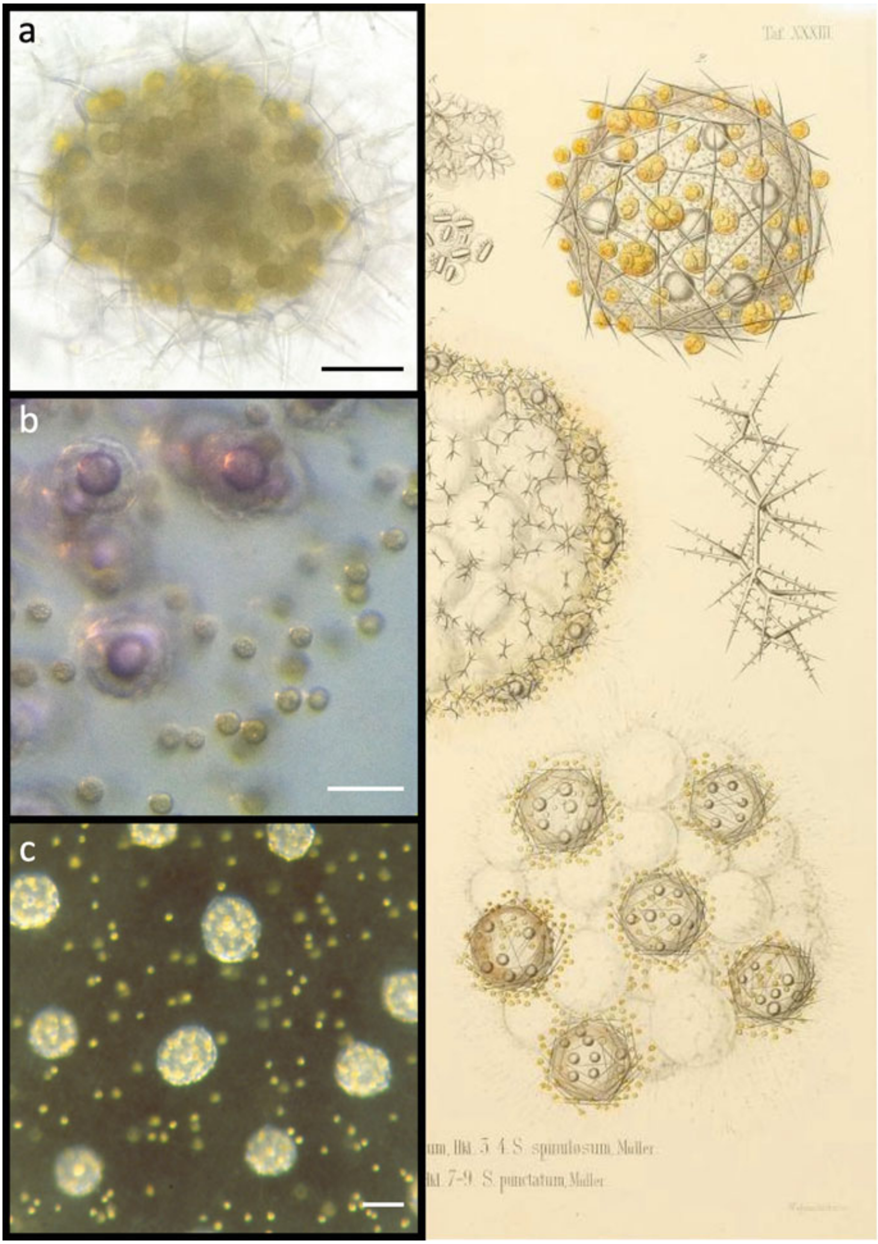

Fig. 13.6 Endosymbiotic algae in association with Radiolaria were documented more than 150 years ago by Ernst Haeckel (1862). The right side of this figure is a portion of a plate from one of Haeckel's highly detailed monographs. The three insets on the left show what Haeckel must have seen through the microscope nearly 150 years ago. Contracted central capsules (not visible) of a spicule-producing colonial radiolarian (**a**) show numerous golden spheres of the dinoflagellate endosymbiont, *Brandtodinium nutricula*, embedded in the pseudopodial matrix around central capsules. Purple central capsules of the colonial radiolarian *Collosphaera* sp. (**b**) contrast with

Light and electron microscopy, and later gene sequencing, helped establish the taxonomy of the dominant algal types that are held intracellularly (i.e., endosymbiotically) within the pseudopodial matrices of planktonic Foraminifera and Radiolaria. A variety of photosymbionts have been documented as associates of Foraminifera (see Table 19.1 in Decelle et al. 2015, Table 1 in Takagi et al. 2019). The most common and best-studied photosymbionts among several spine-bearing foraminiferal species are a single species of dinoflagellate, *Pelagodinium (Gymnodinium) béii* (Siano et al. 2010; Spero 1987). Several non-spinose species of planktonic Foraminifera harbor non-dinoflagellate symbionts (Anderson 2014; Hemleben et al. 1988; Takagi et al. 2019) and at least one spinose species (*Globigerinella (aequilateralis) siphonifera*) harbors either haptophytes or prasinophytes in apparently mutually exclusive symbioses (Faber et al. 1988; Gast et al. 2000). Polycystine Radiolaria also form photosymbioses with a wide array of algal types including prasinophytes, haptophytes, and cyanobacteria (Anderson et al. 1983b; Decelle et al. 2015; Gast and Caron 2001) but the dominant and best-known relationships are formed with a single photosynthetic dinoflagellate species, *Brandtodinium (Scrippsiella) nutricula* (Anderson 1983a, b; Gast et al. 2000; Probert et al. 2014).

The two most common dinoflagellate photosymbionts are morphologically altered within the host cytoplasm. Both *B. nutricula* in Radiolaria and *P. béii* in Foraminifera lose their flagella and thecal plates in the cytoplasm of the host where they are held within perialgal vacuoles produced by the host (i.e., separate from direct cytoplasmic contact with the host, but completely engulfed within the host's cytoplasm). It is presumed although unsubstantiated that these changes are enacted by the host to facilitate molecular communication between host and symbiont and/or the translocation of photosynthate from symbiont to host. The cellular processes bringing about these morphological changes in the photosymbionts are unknown.

The dinoflagellate photosymbionts of planktonic Foraminifera and Radiolaria have been shown to contribute substantively and variously to the nutrition of their hosts. Symbiont abundances within the pseudopodial network remain more or less constant or even increase in number when the hosts are reared in the laboratory and fed prey, and fast repetition rate fluorometry (Fv/Fm) has revealed that the symbionts have high photosynthetic capacity during much of the host's life span (Takagi et al. 2016, 2019), even if a few symbionts are digested along the way (Anderson 1983a). Symbiont persistence has been taken as an indication that these relationships are mutualisms. Symbiont photosynthetic rates have been measured using microprobes to measure oxygen concentrations in response to light and dark (Jørgensen et al. 1985) and by traditional ^{14}C-based measurements (Anderson 1978; Anderson et al. 1983a, 1989; Caron et al. 1995; Michaels 1991; Spero and Parker 1985). These studies have revealed extremely high rates of organic carbon production by the

Fig. 13.6 (continued) the golden *B. nutricula* cells. Central capsules of the colonial radiolarian *Collozoum* sp. (**c**) have golden *B. nutricula* distributed throughout the pseudopodial matrix. Marker bars are all 100 μm symbionts implying that symbiont primary production probably meets most if not all of the energetic demand of their hosts and that feeding by hosts may be as important for obtaining major nutrients, particularly nitrogen and phosphorus, as for organic carbon acquisition. One foraminiferan possessing *P. béii* symbionts, *Globigerinoides sacculifer*, reared in the light without prey remained alive for weeks, although symbiont number gradually dwindled and hosts eventually died without increasing in size (Bé et al. 1981). In contrast, specimens placed in continuous darkness or deprived of photosynthetic production by treatment with DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) died rapidly even in the presence of sufficient prey for foraminiferal growth indicating a strong dependency on the symbionts that might extend beyond energy/carbon requirements (Bé et al. 1982; Caron et al. 1982).

These "simple" experimental studies have revealed that different photosymbiont associates contribute uniquely to the life processes of their hosts. Some polycystine Radiolaria, for example, obtain so much nutrition from their symbionts that they have occasionally been considered phototrophic species because they have little apparent dependency on capturing prey although this generality does not apply to all symbiont-bearing species (Swanberg et al. 1986). In contrast, the foraminiferan *G. sacculifer* appears to be quite dependent on prey for growth as noted above (Bé et al. 1981; Caron et al. 1982; Takagi et al. 2016, 2018). A similar dependency on prey appears to be the case for the foraminiferan *Orbulina universa* despite the fact that it possesses large numbers of highly active dinoflagellate symbionts (Caron et al. 1987; Spero and Parker 1985) (Fig. 13.5d). Symbiont type for a given foraminiferal host also affects the nutritional state of the host. *G. siphonifera* establishes mutually exclusive photosymbioses with either prasinophyte or haptophyte algae, as noted above. The growth and longevity of the host differ depending on which symbiotic alga is present (Faber et al. 1989).

Knowledge of the exact nature of the metabolic coupling between hosts and photosymbionts in planktonic Retaria has been severely technique-limited until recent years, preventing a clearer understanding of the true nature of these symbioses and masking differences among them. Translocation of symbiont photosynthate to host was demonstrated decades ago (Anderson 1978; Anderson et al. 1983a) but progress beyond that basic tenet has been slow. The fragility and lack of amenability of these delicate organisms to laboratory culture have rendered them intractable for some traditional approaches commonly applied to large Cnidaria (Meyer and Weis 2012). The application of gene sequencing (and advanced imaging; see Sect. 13.4.2) to retarian photosymbioses, however, has begun to change that situation because such measurements can be made without the need for extensive handling or culture. The large genomes of these specimens, particularly those of the dinoflagellate symbionts, thus far still thwart the complete sequencing of genomes in most of these symbioses but transcriptomic analyses are providing novel insights into the metabolic interplay between host and symbionts in planktonic Retaria as well as many other ecologically relevant microbial eukaryotes lacking sequenced genomes (Caron et al. 2016).

Transcriptomics has begun to identify the genetic machinery that might be involved in establishing and maintaining retarian photosymbioses, and the impact of the associations on symbiotic algae. One study comparing gene expression of four rhizarian species (three symbiont-bearing Radiolaria and one aposymbiotic cercozoan rhizarian) concluded that c-type lectin-coding genes might be involved in establishing or maintaining photosymbiosis. This conclusion was based on differences in the expression levels of those genes in the symbiont-bearing versus aposymbiotic species (Balzano et al. 2015). Lectins play a role in cell-cell recognition in eukaryotes and therefore may hold clues as to how partners in rhizarian photosymbioses recognize each other.

Comparative studies of gene expression of *B. scrippsiella*, the dinoflagellate symbiont of the radiolarian *Thalassicolla nucleata* (Fig. 13.5e) in both the free-living and symbiotic state have implicated nitrogen transformations and amino acid production as key factors in the chemical interplay between host and symbiont (Liu et al. 2019). Symbiont genes that showed increased transcription in the symbiotic state included several genes involved in nitrogen transport and transformation, while genes involved in RNA and protein synthesis showed decreased transcription (Fig. 13.7). There was no evidence for increased carbohydrate or glycerol metabolism *in hospite* but pathways of amino acid synthesis were enhanced. Collectively, the findings implied a suppression of symbiont growth in the host's cytoplasm and amino acids as a potential form of organic carbon transfer from symbiont to host. Somewhat different biosynthetic pathways for symbiont photosynthate were identified from a study employing NanoSIMS with the planktonic foraminifer *O. universa* and its dinoflagellate symbiont *P. béii*. Starch appeared to be a major form of photosynthate produced by the symbionts during the day with translocation of photosynthate to the host mostly at night possibly in the form of lipids (LeKieffre et al. 2018).

These seminal applications of -omic approaches to retarian photosymbioses have begun to answer some questions regarding the establishment and function of retarian photosymbioses but have also raised other questions as to the presumed mutualistic nature of these relationships. It is clear that photosymbionts provide their hosts with substantial nutrition but a reciprocal benefit to the algae is difficult to argue in some situations. The ultimate fate of the symbionts amplifies this point. The ontogeny of planktonic Retaria involves development from swarmer cells that are a few micrometers in size (cells presumed to be haploid gametes) to adult specimens that may be macroscopic (Anderson 1976; Bé and Anderson 1976; Bé et al. 1983). Adult solitary forms or colonies at the time of reproduction resorb their pseudopodial networks and the entire cell undergoes multiple divisions into thousands to hundreds of thousands of minute flagellated swarmer cells. Symbionts are not transmitted vertically from adult to progeny. Swarmer formation has now been documented for numerous species but little is known and much is speculated regarding the early life stages of these species (Anderson 1983a; Hemleben et al. 1988). The ultimate fate of the symbiotic algae at the time of onset of the host's reproductive process is poorly understood for most species but their fate has great import for understanding whether Retarian photosymbioses are truly mutualistic.

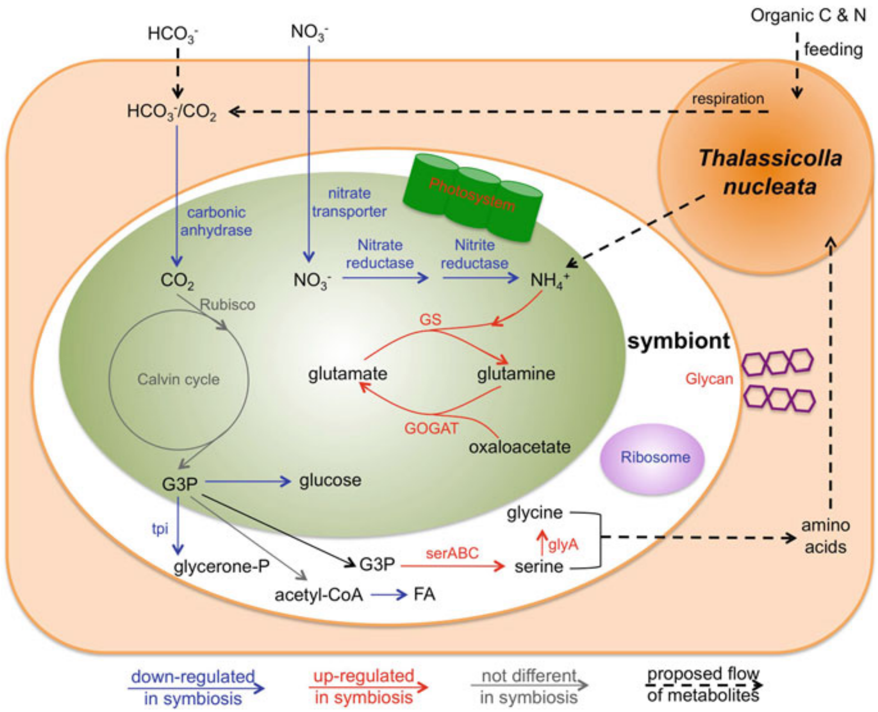

Fig. 13.7 Proposed metabolic interactions between the radiolarian, *Thalassicolla nucleata* and its photosynthetic dinoflagellate endosymbionts, *Brandtodinium nutricula* inferred from transcriptomic data. Metabolic pathways that were up- and downregulated in the dinoflagellate in the symbiotic versus the free-living state are highlighted in red and blue, respectively (Fig. 5 from Liu et al. 2019; see for additional details)

Many Radiolaria appear to expel or discard their dinoflagellate symbiont, *B. nutricula*, at the onset of reproduction. The host's impending swarmer production thereby releases hundreds to thousands of symbionts into the environment, potentially a net "gain" for the dinoflagellate population in the free-living state if they have multiplied in the cytoplasm during the host's ontogeny. It could be argued that such a situation would constitute a mutualistic symbiosis. At least some of the symbionts released at the time of swarmer formation remain viable because they have repeatedly been cultured as free-living dinoflagellates from material discarded when swarmer cells are released. In contrast, thousands of the photosymbiont *P. béii* held in the cytoplasm of adult specimens of the foraminifer *G. sacculifer* are digested en masse at the onset of the host's reproductive cycle (Bé et al. 1983). A similar fate appears to be the case for several other Foraminifera that harbor *P. béii* and *G. siphonifera* which harbor non-dinoflagellate symbionts (Takagi et al. 2016). These findings imply an association that is less than mutualistic, and that the algae are merely 'farmed' to serve as an energy source for the energetically demanding process of cellular reorganization and division of the host's cytoplasm that characterizes reproduction in these Foraminifera. While symbionts are beneficial to the nutrition and life processes of the host, the alga ultimately gains nothing from the association if the symbionts are eventually digested.

The apparently disparate fates of symbionts in radiolarian and foraminiferal hosts raise questions with respect to whether it has been accurate to assume that all (or most) retarian photosymbioses are mutualistic relationships. Recent findings described below, obtained with cutting-edge imaging and -omic approaches, have begun to demonstrate a situation in the well-known Acantharia-*Phaeocystis* photosymbiosis that is far from mutualistic, where the relationship has clearly crossed the line from mutualism to symbiont "farming" or perhaps something even closer to organelle acquisition.

13.4.2 Photosymbioses, Organelle Acquisition, and the Acantharia–Phaeocystis Symbiosis

Early classifications of single-celled eukaryotes separated protists into two groups based on trophic modes: photosynthetic forms (algae), and heterotrophic forms (those that consume prey or other preformed organic matter: protozoa). There are, of course, examples of species that exhibit only phototrophy (e.g., many diatoms) or only heterotrophy (e.g., many ciliates), but modern protistologists (and their many phylogenetic revisions of the last few decades) recognize that trophic mode is a poor indicator of evolutionary relationships or ecology among these species. Many, perhaps most, planktonic protists exhibit a mixture of these fundamental trophic modes and the term "mixotrophy" has come into common use to describe individual species or pairs of species that exhibit a combination of photosynthetic and heterotrophic abilities (Flynn et al. 2019; Stoecker et al. 2017). The descriptor mixotrophy, therefore, encompasses many photosynthetic eukaryotes that possess their own chloroplasts (i.e., true eukaryotic algae) but also possess the ability to consume and digest prey as well as situations where heterotrophy and photosynthetic abilities originate from two different species. These mixotrophic capabilities have considerably changed the way we envision pelagic food web structure and biogeochemical cycles (Mitra et al. 2014; Stickney et al. 2000; Ward and Follows 2016).

The situation of heterotrophic hosts with photosynthetic symbionts is exemplified by many planktonic Retaria (Figs. 13.5 and 13.6). Such relationships cover a spectrum of interactions from the apparently mutualistic endosymbiosis detailed above for many Radiolaria, algal 'farming' noted for at least some planktonic Foraminifera, to the reduction and retention of specific organelles of photosynthetic prey ingested by heterotrophic protists (in the case of chloroplast retention this ability is often called kleptochloroplastidy). Numerous examples of the latter behavior exist across the heterotrophic protistan lineages most notably among planktonic retarians as described above, ciliates (Dolan 1992), and dinoflagellates (Stoecker 1999), but extending even to some flatworms (Stoecker et al. 1989) and molluscs (Hinde and Smith 1974). These interactions do not entail complete integration of the symbionts or their organelles into the host's metabolism, physiology, and cell cycle (a situation that characterizes true organelles) but they are also not mutualistic interactions as has often been assumed.

The relationship between some species of cryptophyte algae and species of the ciliate genus *Mesodinium (Myrionecta)* has become a well-studied system for investigating the one-sidedness that can exist among some protistan associations formerly assumed to be mutualistic photosymbioses (Johnson et al. 2017). Advanced imaging and genetic studies have begun to characterize the mechanism of algal capture, partial digestion, and organelle retention that results in the acquisition of functional chloroplasts for the heterotrophic/mixotrophic *Mesodinium*. Electron- and fluorescence microscopy (the latter enhanced with fluorescent in-situ hybridization (FISH) probing) has documented substantial reduction of the endosymbionts but retention of the cryptophyte's chloroplasts and nuclei, which remain transcriptionally active and serve to maintain chloroplast function in the host cytoplasm, endowing the host with a substantial photosynthetic ability (see Fig. 1 in Johnson et al. 2007).

Subsequent genomic and transcriptomic studies have detailed major changes in the transcriptional activity of the acquired cryptophyte components (Altenburger et al. 2021; Lasek-Nesselquist et al. 2015). Cryptophyte genes associated with photosynthesis, carbohydrate biosynthesis, and amino acid biosynthesis show enhanced transcription in *Mesodinium* containing sequestered and reduced cryptophytes, relative to free-living cryptophytes. The extensive metabolic rewiring of the cryptophyte organelles persists for weeks although chloroplast function eventually breaks down and new "symbionts" must be ingested.

The cryptophytes in the *Mesodinium*-cryptophyte interplay do not benefit nor do they survive these extraordinary alterations. As a consequence, the relationship is far from a mutualistic association, or even "farming" of algae as in the situation described above for the foraminifer *G. sacculifer* but appears to be rather far along an evolutionary pathway to permanent tertiary endosymbiosis (i.e., true organelle acquisition). The cercozoan *Paulinella chromatophore* and its cyanobacterial chromatophore noted above (Sect. 13.2.3) constitute a relationship that apparently is even farther along the path from mutualistic interaction between symbiotic partners to organellogenesis (see Fig. 1 in Nowack and Melkonian 2010). A greatly reduced genome of the cyanobacterium and substantive protein trafficking in *P. chromatophora* has been taken as evidence of an evolutionary progression towards photosynthetic organelle acquisition by a "heterotrophic" protist (Nowack and Grossman 2012; Singer et al. 2017).

The examples above illustrate a spectrum of symbiotic interactions ranging from truly mutualistic to nearly complete organelle acquisition. Nonetheless, there has been a persistent preconception that retarian photosymbioses are mutualisms. This notion has changed only slowly, in part as a result of studies of the photosymbiosis involving radiolarian Acantharia and their preferred symbiotic algae (species of the haptophyte genus *Phaeocystis*; Fig. 13.8) (Decelle et al. 2012). Recent studies have revealed similarities to aspects of the ciliate-cryptophyte association noted above through the application of advanced microscopy, isotopic elemental tracing, and

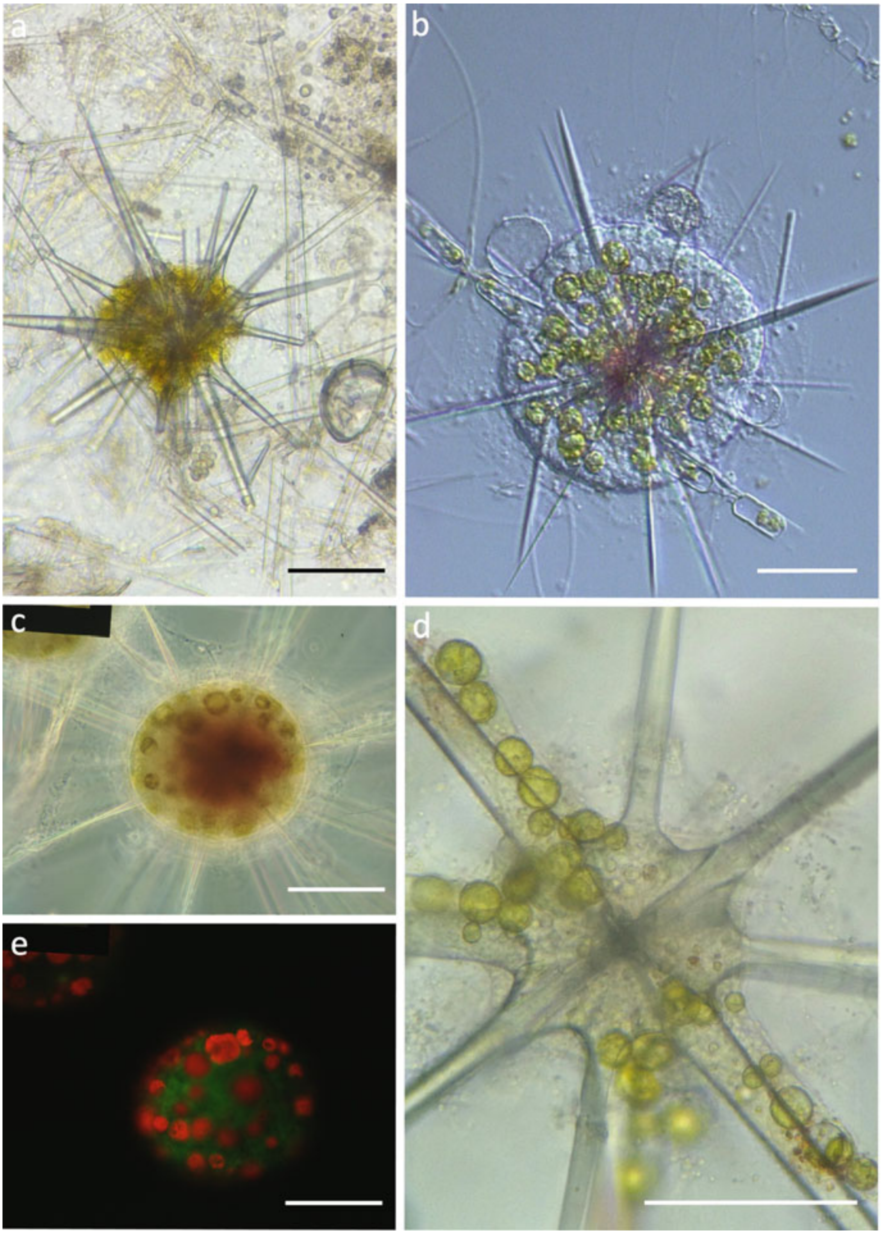

Fig. 13.8 Many Acantharia establish photosymbiotic associations with species of the haptophyte genus *Phaeocystis*. Endosymbionts in these micrographs appear as yellowish spheres within the cytoplasm of the acantharian hosts. Light micrographs of compressed specimens of Acantharia reveal the presence of the closely-held symbionts (**a**, **b**). A light micrograph (**c**) and an epifluorescence micrograph of the same acantharian cell helps visualize the symbionts, the latter are autofluorescent with blue light excitation due to the presence of chlorophyll *a*. Algal cells of different sizes can be present in a single host (**d**), presumably an indication of the degree to which the algal architecture has been altered (see Decelle et al. 2019). Marker bars are all 100 µm

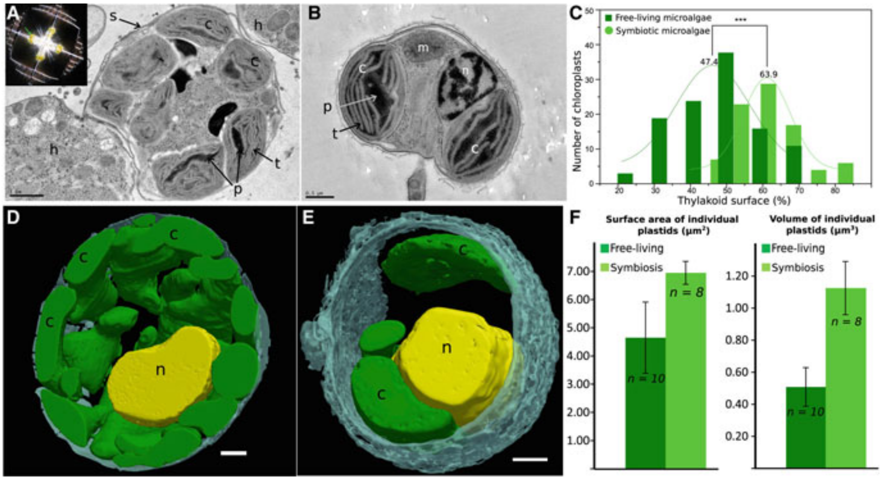

Fig. 13.9 Transmission electron micrographs of *Phaeocystis cordata* in the symbiotic state (**a**) and cultured as free-living cells (**b**) showing marked differences in symbiont size and chloroplast number, with supporting data (**c**). Symbionts are highly modified in the symbiotic state to enhance photosynthetic capacity. Focused ion beam scanning electron microscopy of the same pairing (**d**, **e**) and supporting data (**f**) provide striking visualization of the differences in these features. The inset in (**a**) shows a low magnification micrograph of the acantharian host and its ornate skeleton (Fig. 1 from Decelle et al. 2019; see for additional details)

extensive genetic analyses (see Fig. 2 in Decelle et al. 2020) that have helped characterize the activities and ultimate fate of the photosymbionts.

Free-living *Phaeocystis* cells are transformed remarkably, and apparently irreversibly, when they are taken into the acantharian cytoplasm in this photosymbiosis. Decelle et al. (2019) reported that the volume of the haptophyte chloroplasts was dramatically increased (up to tenfold) and photosynthesis was enhanced accordingly (Figs. 13.8b,d and 13.9). Transcriptomic information was also obtained for this photosymbiosis. The analyses indicated that symbiont cell division was prevented, and expression of photosynthetic carbon fixation pathways was increased many-fold in the cytoplasm of the host (Uwizeye et al. 2020). Notably, the authors concluded that changes in the symbionts were so extensive that it is highly unlikely that they could reestablish themselves as free-living algae were they released from the host, meaning that this photosymbiosis is not at all a mutualism but rather a farming strategy, or “cytoklepty,” with the host commandeering the photosymbiont for its own gain (Uwizeye et al. 2020). Their speculation of irreversible changes in the symbionts is supported by the anecdotal finding that “symbiotic” *Phaeocystis* has yet to be successfully cultured as free-living cells from the photosymbiosis.

These exciting new findings that retarian photosymbioses actually represent a wide spectrum of species-species interactions from mutualism to cytoklepty has opened interesting avenues of research regarding how and how fast ‘acquired phototrophy’ has evolved in planktonic Retaria, and other heterotrophic protists. The time frame for these transformations is the subject of much debate and research.

Similarly, the series of events that give rise to effective cross-talk between symbiont and host (and therefore host control of the symbionts) is still poorly constrained. Are the events that lead to eventual gene relocation and integration of the former-symbiont-now-plastid into the host genome rapid and fortuitous or slow and methodical? Both seem possible based on our current knowledge of horizontal gene transfer.

Questions also revolve around how algal species are chosen as photosymbionts. Are certain algal species more susceptible to establishing symbioses? A provisional answer to that question appears to be "yes" at least for some algae. *B. nutricula* establishes endosymbioses with numerous Radiolaria as well as some pelagic invertebrates, and *P. béii* is the preferred symbiont of several Foraminifera (Gast and Caron 1996, 2001). *Phaeocystis* species are commonly encountered as endosymbionts in several Acantharia, but retention of the plastids of these algae has also been observed in a common Antarctic heterotrophic dinoflagellate (Gast et al. 2007). These findings imply that some algae are more amenable to establishing and maintaining symbiotic associations (Gomaa et al. 2014), although the genetic bases for this ability are only slowly coming into focus (Nowack and Melkonian 2010). Photosymbioses in planktonic Retaria have proven informing yet challenging subjects with which to address these questions because they offer a wide range of endosymbiotic associations to explore.

13.4.3 Parasitic Symbioses Involving Planktonic Retaria

Morphological and physiological modifications of algal symbionts as described above represent a situation in which heterotrophic hosts commandeer their photosynthetic prey (or their organelles) but there are also situations in which protists infect and kill larger planktonic Retaria. Ectoparasites of planktonic Retaria have rarely been observed in part because of the difficulties of capturing and maintaining these specimens in the lab and in part because the pseudopodial networks of these specimens are exceedingly sticky, ensnaring and digesting most species that come in contact with them (Anderson 1993). Nonetheless, a few ectoparasites of planktonic Foraminifera have been documented and include a number of heterotrophic gymnodinoid and peridinoid dinoflagellates (see Fig. 5.4b in Hemleben et al. 1988). These species navigate the complex array of spines and pseudopodial networks of their hosts with apparent impunity, darting between pseudopodia-laden spines to occasionally remove and ingest bits of the host cytoplasm. Planktonic Retaria do not appear to be infected by many of the phylogenetically diverse endosymbiotic parasites common among phytoplankton taxa (e.g., chytrids, oomycetes; see Sect. 13.2.3).

Radiolaria and possibly Foraminifera are, however, hosts to dinoflagellate-like alveolate (MALV) groups (Coats 1999; Guillou et al. 2008) that possess heterotrophic parasitic lifestyles. These species "turn the tables" on their hosts relative to the outcomes of most of the photosymbioses described above. Life cycles of only a few of these parasites are well-known (e.g., *Amoebophrya*, *Parvilucifera*). They are commonly encountered in coastal plankton communities where it has been documented that they can considerably affect the abundances of their hosts (Alacid et al. 2015; Cachon and Cachon 1987; Chambouvet et al. 2008; Coats and Heisler 1989). The life cycle of these species involves entry into the host by motile zoospores, escape from the digestive processes of the host, growth and cell division inside the host by feeding on its cytoplasm, and subsequent release of the zoospores resulting in lysis and death of the host (see Fig. 1 in Alacid et al. 2015). The process is roughly analogous to viral infection, replication, and host lysis except that exploitation of the host's genetic machinery has not been documented among parasitic protists to our knowledge.

Awareness of parasitic protists in coastal ecosystems has been known for many years, including knowledge of some parasitic dinoflagellates associated with Radiolaria (Hertwig 1879). However, understanding the ubiquity and huge diversity of parasitic protists, and recognition that many have retarian hosts, has been enhanced greatly through gene sequencing surveys of environmental samples. Early genetic studies of parasitic symbioses between planktonic Retaria and alveolate parasites employed gene sequencing to draw the connection between the partners (Bråte et al. 2012; Dolven et al. 2007; Gast 2006; Guillou et al. 2008). Global surveys have used gene sequencing and network analysis to identify the diversity and putative links between parasites and retarian hosts (Lima-Mendez et al. 2015). Networking approaches have also been applied to understand linkages between specific retarian hosts, such as the planktonic foraminiferan *Neogloboquadrina pachyderma* and alveolate protists that may parasitize it (Greco et al. 2021). The transcriptomic study of (Liu et al. 2019) described above to characterize the photosymbiosis between the radiolarian *T. nucleata* and its dinoflagellate prey *B. nutricula* revealed the presence of a dinoflagellate-like, putative parasite alveolate in addition to the photosymbiont (Liu et al. 2019). Beyond these initial insights into parasitic symbioses involving planktonic Retaria largely open questions remain regarding host-parasite specificity, the contribution of alveolate parasites to host mortality, and the potential for these associations to alter retarian community structure in nature.

13.5 Concluding Remarks: Potential Scientific and Technological Benefits of Understanding Symbiosis

Innovations facilitated by symbiosis have played central roles in evolution enabling the evolution of eukaryotic respiration and photosynthesis and the subsequent enormous diversification of life. In addition, symbiotic associations continue to comprise major drivers of ecological responsiveness of species in the face of environmental change. The vast microbial diversity in the environment, including the marine environment, has been recognized in recent decades partially spawned by the revolution in molecular biology and genomics. New DNA sequence knowledge has dovetailed with classical and new observations to uncover previously unknown microorganisms, including interactions and symbioses. Surprising discoveries of novel microorganisms and the types of interactions among microorganisms suggest there are many more discoveries of symbiosis yet to be made in the ocean plankton.

The spectrum of symbiotic associations discussed in this review demonstrate a continuum of relationships ranging from independent free-living species to true organellogenesis are providing insight into how and why symbiotic relationships have evolved. New visualization, isotopic, and genetic approaches are facilitating a mechanistic understanding of how microbial symbioses function (Egan et al. 2020). In addition to understanding the diversity of metabolic interconnections between species, research on microbial symbiosis is poised to address even bigger questions with implications for evolution. What are the timescales of the evolution of new symbioses and organisms? What are the cellular and molecular steps leading from loose interactions, to cooperation and dependence in obligate symbioses and organelle evolution? What are the steps leading to gene exchange and protein translocations?

Understanding the processes and mechanisms involved in microbial symbioses is not just of significance to intellectual curiosity but has implications for understanding and predicting the real world at large scales. In addition to providing an understanding of the evolution of biological interactions, and evolutionary processes on Earth and perhaps elsewhere, symbioses play critical roles in biogeochemical cycles and food webs and need to be included accurately in predictive global biogeochemical models. Furthermore, microbial photosymbiosis and N_2-fixing symbioses provide key models that could lead to improvements in photosynthesis and N_2 fixation in agriculture.

It is an exciting and important time in microbial symbiosis research. This review provides only a glimpse of the exciting new understanding that has arisen in the last decade, and hopefully, will foster more research and appreciation of the complexities and importance of marine symbiosis.

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