



Analyzing Coral Reefs and Their Microbial Assemblages

Coral reefs harbor *Symbiodinium* algae and prokaryotic microbes that contribute to host physiology, and pathogens that can undermine it

Mónica Medina

Coral reefs harbor the largest biodiversity of any marine environment on Earth. Regrettably, these reefs now face threats of anthropogenic origin at global and local scales.

At the center of these ecosystems lies a symbiotic partnership, in which coral animals and algal symbionts share responsibilities for constructing and maintaining reef structures. Adult reef-building corals, in general, consist of multiple clonal polyps that take centuries to grow, producing calcium carbonate skeletons that are the framework for the reef ecosystem. Within the coral tissue are unicellular dinoflagellate algae of the genus *Symbiodinium*, commonly known as zooxanthellae (Fig. 1). These dinoflagellates are facultative endosymbionts that provide their coral hosts with nutrients. In turn, the coral provides a sheltered, light-rich environment, as well as inorganic nitrogen and carbon to the dinoflagellates. Additionally, prokaryotic microbes are also thought to contribute to coral physiology, fixing nitrogen, cycling sulfur, and producing antimicrobial compounds. Meanwhile, other microbes are implicated as pathogens in an expanding number of coral diseases.

The *Symbiodinium* algae that coexist with corals and other marine organisms such as sponges and mollusks were thought to be a single species, *Symbiodinium microadriaticum*. However, during the past two decades, molecular systematics studies helped to uncover a large diversity of *Symbiodinium*, which are classified into clades that were named in

alphabetical order as they were discovered. There are now approximately 10 major *Symbiodinium* clades, each having large numbers of species, and ongoing surveys will soon add new clades to that list.

Different coral hosts harbor different symbionts, and the broad range of *Symbiodinium* species provides an extensive physiological repertoire that enables them to cope with varying temperature and light regimes. However, environmental fluctuations are becoming increasingly more extreme. For instance, higher average sea surface temperature (SST) persist for ever-longer periods, raising concerns about how coral-*Symbiodinium* partnerships and, with them, coral reefs will fare.

Summary

- Coral reefs, which harbor rich assemblages of algal and microbial symbionts, are threatened by global warming and other environmental disturbances.
- The onset of the coral-algal symbiosis usually begins during the later stages of embryonic development.
- Rising sea surface temperatures can stress coral, causing breakdown of the coral-algal symbiosis.
- The full microbial assemblage, called the coral holobiont, encompasses a wide range of taxa, including numerous microbial eukaryotes, archaea, bacteria, and viruses.
- Coral hosts likely began to associate with algal and microbial symbionts when the first reefs appeared 240 million years.

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Medina: Former Fencer Now Fancies Ceramics, Microbial Assemblages of Coral Reefs

A native of Colombia, Mónica Medina grew up thinking she would become a medical doctor, like her father. But Rafael Medina apparently knew his young daughter better than she did. “He was the one who led me to look at biology as a career option,” she says. “He was able to understand . . . that the path I wanted was in research, rather than in the clinic.”

Both parents encouraged Medina and her two siblings to be diligent learners, and her brother is an industrial engineer, while her sister became a doctor. “My father gave me many readings in science and literature,” she says. “My mother was always present to make sure we worked hard. My grandparents had a farm where we spent weekends and long periods during breaks. Those things combined probably influenced my interest in learning, and a respect for nature.”

Today Medina, 42, is associate professor in the School of Natural Sciences at the University of California (UC), Merced. Her research focuses on the dynamics of coral and its microbial assemblage under natural and stressful conditions. Understanding the genetic basis of how corals respond to such pressures is essential, and could lead to ways to improve management and inspire mitigation strategies, she says. “Coral reefs are threatened ecosystems due to increasing anthropogenic pressures ranging from global warming and ocean acidification to coastal development and overfishing. I think society is not paying enough attention. Reefs are disappearing fast, and the mounting pressure is only going up.”

Medina and her family lived in Cali, a large city in the south of Colombia. Early on, she nurtured interests in molecular biology and

genetics. “I was very intrigued by gene therapy, which was almost science fiction when I was a kid,” she says. However, she also was developing a love for the natural sciences, especially the ocean. “I was an avid admirer of nature documentaries, especially marine sciences,” she says. “Cali is two hours away from the ocean, and we would go some weekends to the Pacific, but I think my interest for the ocean grew after I saw the beautiful waters of the Caribbean Sea in the north of the country when I was about 10 or 12 years old.”

Her father suggested that she attend Universidad de Los Andes, in Bogotá, where she majored in biology and, in 1989, earned a B.S. degree. “It was a good choice because our professors always encouraged the students to find research opportunities abroad, as there were very few available in Colombia at the time,” she says. Before getting a doctorate, she held a number of research assistant positions, including a two-year internship in Panama at the Smithsonian Tropical Research Institute studying marine taxa, an experience she describes as “transforming.”

Medina earned her Ph.D. in marine biology and fisheries in 1998 from the Rosenstiel School of Marine and Atmospheric Science at the University of Miami, and served two postdoctoral fellowships, first at the Marine Biological Laboratory (MBL) at Woods Hole, Mass., followed by the California Academy of Sciences. At MBL, she gained a deeper appreciation for biodiversity and microbial evolution, and was exposed to genomic analyses for the first time. This, she says, helped her land a job in 2001 at the Department of Energy Joint Genome Institute at Lawrence

Berkeley National Laboratory, where she received her first grant to study coral species.

“Watching corals spawn while a volunteer diver made me want to work on corals,” she says. “Although I did not do my Ph.D. on coral reef science, I’d always hoped the opportunity would present itself. That happened through an idea that was funded by the National Science Foundation Genome Enabled Biocomplexity program in 2003.”

Medina first came to the United States in 1988, and recently became a citizen. “Of course it took a long time to adjust to the U.S. and a new language, and sometimes I am still surprised, but overall it has been a very positive experience,” she says. “This country has given me many opportunities.”

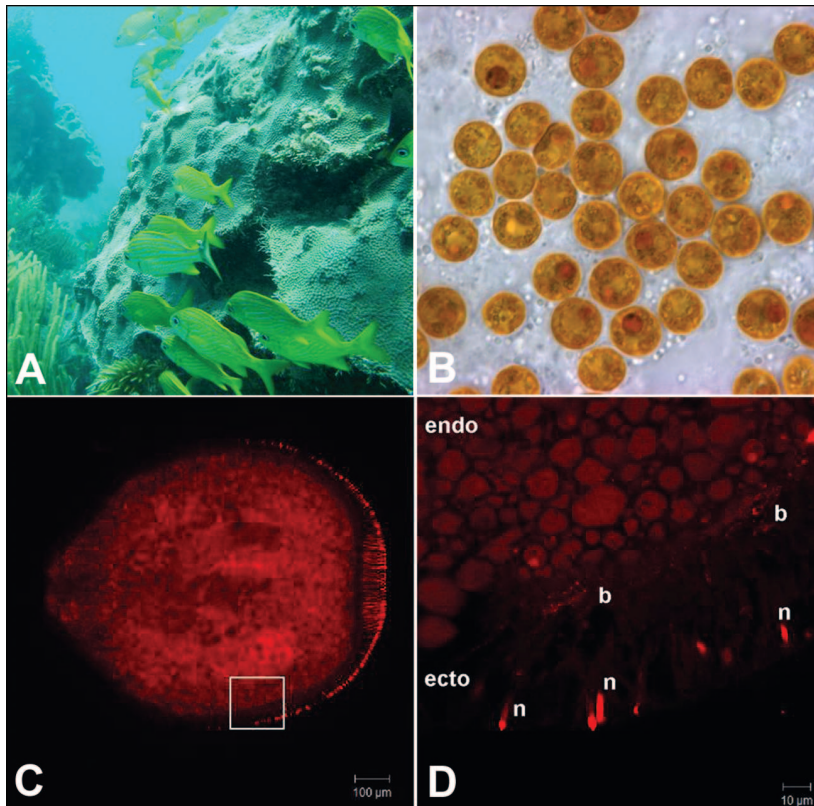
Medina is married to Benoît Dayrat, an evolutionary biologist, who also is on the faculty at UC Merced. They have two young daughters, a toddler and a kindergartener. A competitive fencer since age nine, Medina traveled overseas for her mother country’s national team. “That was really fun, but funding was really tight for women’s sports then and I decided it was best to focus on my studies,” she says. “Right now, there is no fencing near where I live, so I don’t practice the sport anymore,” she says. She also enjoys ceramics, which she practiced for several years, although “right now, I have little time for it,” she says. “I guess my current interests outside work are mainly my children. That leaves little time for much else.”

Marlene Cimons

Marlene Cimons lives and writes in Bethesda, Md.



FIGURE 1



(A) Caribbean reef. (B) *Symbiodinium* cells in culture. (C) Bacteria in *Porites astreoides* planulae. Optical cross section of a newly released *P. astreoides* planula (within 15 min of release from adult colony), hybridized with a CY3-EUB338 (general bacterial probe). (D) High-magnification image of the boxed region in panel C. B (b) are visible in the base of the ectoderm. Also visible in the image is nonspecific probe binding to nematocysts (n) in the larval ectoderm. ecto, ectoderm; endo, endoderm. (Photo credits: A, Mónica Medina; B, Todd Lajeunesse; C and D, Koty Sharp.)

Coral Life Cycles, Onset and Breakdown of Dinoflagellate Symbioses

Coral reproduction is highly intertwined with algal symbiosis. Coral species reproduce both asexually and sexually, which can entail either internal or external fertilization. After fertilization, embryonic development leads from a blastula to a gastrula stage, during which the planula larvae form, the only motile stage.

Planulae swim actively, enabling them to settle on substrates such as coral rubble that typically are covered by crustose coralline algae (CCA). These algae are linked to chemical cues that induce larval settlement and metamorphosis. The sessile polyp develops two tissue layers, an ectodermal layer and a gastrodermis, and

starts producing the first skeletal calice. Onset of *Symbiodinium* symbiosis usually begins during these later stages, although earlier embryonic stages can also be colonized (Fig. 2).

Species that are internally fertilized are called brooders because embryonic development takes place inside the parent polyps, which release fully developed larvae seeded with vertically transmitted *Symbiodinium*. Thus, brooding species tend to harbor the same type of algal symbiont as the parent colony. Free-spawning corals often produce large amounts of gamete bundles that are seasonally released into the water column. Coral mass spawning, which is linked to seasonal warm waters and lunar cycles, happens at different times of the year in different oceans. A large fraction of coral species is made up of spawners acquiring their *Symbiodinium* by horizontal transmission. This mode of reproduction allows the host to take up diverse assemblages of algal symbionts. Coral larvae, in general, have less restricted preferences in terms of algal types than do adults of the same species.

Asexual reproduction takes place by budding and fragmentation. A polyp starts to divide by budding days after settlement. Even if the initial algal complement is diverse, a winnowing process leads to a simpler *Symbiodinium* composition in adult colonies. Moreover, strong waves can fragment corals, leading to new colonies of clonal origin that will have the same *Symbiodinium* complement as the parent colony.

Coral Bleaching Involves Breakdown of Symbiosis

Coral bleaching, the breakdown of the coral-algal symbiosis, is now widespread. Although several environmental factors can induce bleaching, rising sea surface temperatures, exposing reefs to prolonged periods of thermal stress, are the most alarming in terms of impact.

Disruption of the coral-algal symbiosis can prove fatal for the host, leading to major losses in coral cover after extensive warm periods. For



example, anomalously high sea-surface temperatures in the Caribbean during the summer of 2005 triggered the largest bleaching event recorded in 50 years. During 2010 even longer thermal anomalies caused massive bleaching in many reef locations around the globe, but a complete assessment of coral losses from last year is not yet available.

After a bleaching event, adult colonies can switch proportions of *Symbiodinium* types or take up more thermotolerant symbionts (Fig. 2). These replacement *Symbiodinium* species typically belong to clade D, although in the Caribbean, clade A symbionts also tend to outcompete other algal types shortly after thermal stress. After observing bleaching-induced symbiont switching, some scientists were hopeful that *Symbiodinium* clade D would enable coral reefs to cope with warming oceans. However, new data suggest that these algae tend to be more physiologically costly for hosts to maintain, and the hosts tend to revert to their original algal complement once their thermal stress is alleviated. Thus, propagating coral fragments with thermotolerant symbionts or seeding bleached corals with clade D *Symbiodinium* species may not protect coral reefs against global warming.

The Coral Holobiont: a Succession of Microbial Assemblages

Decades of research provide insight into how the coral-algal symbiosis is established and maintained as the host ages. However, little is known about how other microbes come to associate with corals and of their subsequent dynamics. Yet, coral species harbor diverse microbial species extending far beyond a few *Symbiodinium* phylotypes. This microbial assemblage, referred to as the coral holobiont, encompasses a wide range of taxa, including numerous microbial eukaryotes, archaea, bacteria, and viruses. Although in its infancy, the study of coral-associated microbial eukaryotes already identified several species of potentially beneficial fungi, ciliates, alveolates, stramenopiles, and other algae. These organisms apparently contribute to the overall health of their coral hosts.

Genomics and related techniques are providing insights into how coral hosts respond to different

Table 1. Key questions in coral microbiology

- Have coral microbial holobionts co-evolved with their coral hosts? Can we detect this process with current methods?
- Is the specificity of core holobiont microbiomes due to coevolution or simple co-diversification after host speciation events?
- What are the spatial and temporal dynamics of coral microbial assemblages?
- How is horizontal gene transfer shaping the coral holobiont?
- How do microbes contribute to coral health in general?
- What do microbes contribute to the success of corals living in nutrient poor environments?
- What are the mechanisms by which corals develop antimicrobial resistance?
- What are the specific and overall metabolic potentials of microbes in terms of carbon and nitrogen fixation?
- How will shifts in taxonomic composition of microbial communities translate into overall functional changes?
- What are the host and *Symbiodinium* functional responses to shifts in microbial diversity?
- Is habitat complexity proportional to microbial diversity?
- How do environmental and ontogenetic changes affect the microbial assemblages in the coral holobiont?
- What is the role of the microbial assemblages in the onset and propagation of coral diseases?
- What is the intra-individual (within one colony), inter-individual (between colonies of the same species), and interspecific (between different species) variability of bacterial communities in corals?
- What is the temporal variability of bacterial communities in coral individuals and populations?
- Which are the key ecological factors that drive regional differences in bacterial community composition?
- Are disease-causing agents already present in healthy corals, e.g., as commensals or dormant opportunists?

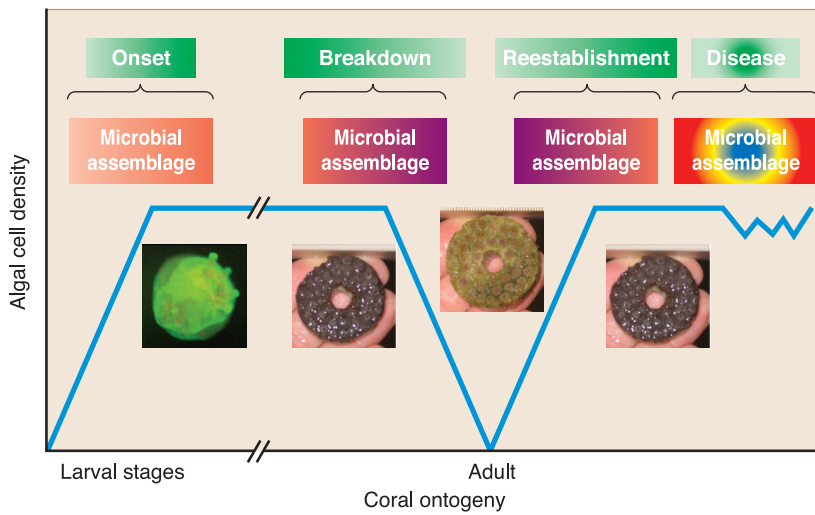
algal complements. For instance, using gene expression microarrays, my collaborators and I learned that exposing coral hosts during early larval stages to incompetent *Symbiodinium* species—those that fail to infect their hosts—elicits major transcriptional responses. However, competent *Symbiodinium* species enter the larvae in a stealthy manner, yielding transcriptional profiles that resemble those of larvae that are not exposed to *Symbiodinium*.

Transcriptional profiles change in thermally bleached fragments of *Montastraea faveolata*. Those changes appear to correlate with symbiont switching. Although coral species typically harbor only one type of *Symbiodinium*, *M. faveolata* harbors many types. We still do not know how a coral host, monotypic for *Symbiodinium*, responds to thermally induced bleaching at the transcriptome level.

Sequencing of clone libraries for the small ribosomal subunit RNA gene (16S) hinted at large pools of microbial diversity in corals. More recent efforts add severalfold increases to those estimates. Genome-enabled analysis also



FIGURE 2



Shifts in the microbial assemblage through the ontogeny of the coral host. The Y-axis represents the changes in abundance of *Symbiodinium* over time, which is here used as a proxy for shifts in health state. Shifts in *Symbiodinium* content are also depicted by the green boxes above the curve. Changes in microbial community composition are depicted by the orange-purple boxes. The microbial community is most diverse in disease states and is represented by multiple colors in the corresponding box. (Photo credits: *M. faveolata* larva with *Symbiodinium* cells, Alina Szmant; coral fragments, Michael De-Salvo.)

prompts a major leap forward in terms of uncovering microbial diversity in coral reef ecosystems. For instance, we know that some microbial species in the coral mucus have antimicrobial activity, but the active compounds are not yet identified. The mucus layer appears to harbor the most microbial diversity, but microbes are also found inside host tissues as well as underneath the skeleton-secreting tissue layer.

It will be important to understand the role of specific microbial species in biogeochemical cycles. Metagenomic and qPCR-based approaches combined with culture-independent technologies such as single-cell genomics will help these efforts. High-resolution fluorescent in situ hybridization and scanning electron microscopy approaches are shedding light into where and when microbial species are established in host corals.

Succession of Microbial Assemblages

Because different reproductive strategies lead to recruitment of different *Symbiodinium* symbionts, establishing microbial assemblages during early stages of coral development may lead to

patterns that reflect those different modes of reproduction. In the few free-spawning and -brooding taxa examined so far, microbial species seem to be recruited from the water column during the early embryonic and larval stages, as opposed to being vertically transmitted by the parent colony. According to Nicole Webster and colleagues at the Australian Museum of Marine Science, once the planulae are competent for metamorphosis, the microbial biofilm on the CCA plays a critical role in driving larval settlement rates. Moreover, increasing the SST a mere 2–4°C above the ambient mean temperature has major detrimental effects on coral recruitment.

In the case of seeded larvae, prevalent *Symbiodinium* associations seem to play a critical role in what microbial taxa are recruited into the developing stages. In thermotolerant *Symbiodinium* species, these associations can exert harmful downstream effects on coral hosts. For instance, according to Raechel Littman and colleagues at

James Cook University and the Australian Institute of Marine Science, coral larvae seeded with *Symbiodinium* clade D (a thermotolerant species) under similar thermal stress, shifted from one microbial complement to another. When compared to larvae seeded with naturally occurring *Symbiodinium* clade C, the clade D-seeded larvae not only showed declines in physiological performance but also exhibited higher levels of potential pathogenic bacterial strains such as *Vibrio*.

The microbial community in adult-stage coral can fluctuate along seasonal and biogeographic ranges. For instance, the genetic diversity of the coral populations can be tightly coupled with the *Symbiodinium* assemblages that inhabit them. Microbial diversity shifts at biogeographic scales may also be associated with the genetic population structure of the host-algal assemblage.

Pollution gradients also shape coral microbial communities, and shifts in microbial diversity correlate with coral health states. Bleaching or diseases can cause dramatic changes in corals. In diseased corals, microbial diversity tends to increase, encompassing recognized pathogens.



Thus, microbial community composition seems to play an important role in coral health, starting with the early microbial assemblage.

Coral Species May Have Coevolved with Its Microbes

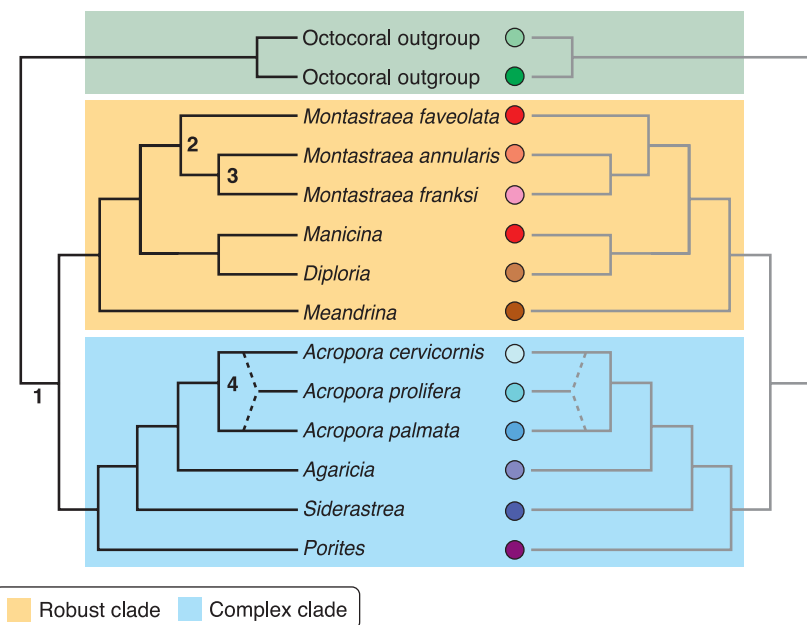
Our analysis indicates that microbes associated with congeneric coral species are more alike than are those associated with more distantly related corals. Thomas Bosch and his group at the University of Kiel in Germany, observed similar patterns among microbial assemblages in *Hydra*, another cnidarian lineage, which hints at coevolution between the host and its associated microorganisms. Thus examining an evolutionary scenario in which coral hosts may have begun to associate and coevolve with specific microbial communities when the first reefs appeared 240 million years ago (Fig. 3), seems a worthwhile endeavor.

If microbial assemblages reflect diversification among corals, further analysis may enable us to detect cospeciation events and to reconstruct the composition of the ancestral coral holobiont. With the coral fossil record, such findings could help in reconstructing the role of microbes throughout the history of coral reefs. Although other processes are likely to affect the microbial composition of the coral holobiont, a phylogenetic framework provides an important launching point for this analysis.

The genetic makeup of coral algal symbionts appears to define population and species boundaries in some coral taxa. If core sets of microbial species are coevolving with their coral hosts, then the genetic makeup of these microbial assemblages likely also has an impact on host fitness. Microbial symbiosis may provide the means to cope with environmental insults brought on by climate change. Changes in these microbial communities could occur on ecological time scales, potentially enabling particular coral holobionts to survive environmental changes. Testing these hypotheses will depend on first characterizing the coral microbiota.

In addition to coevolution, coral colonies face an eclectic and random mix of microbes through

FIGURE 3



A coevolutionary hypothesis for corals and their associated microbial assemblages. The coral phylogeny depicted in black is well supported by molecular data (node 1 = *Scleractinia*). The hypothetical distance based on microbial community compositions is depicted in gray on the right side. Recent data have shown more similar microbial communities in congeneric species with short divergence times such as the *Montastraea annularis* species complex (nodes 2 = 6.5–5.6 Ma and 3 = 0.5 Ma) and the *Acropora palmata* and *A. cervicornis* complex (node 4 = 5.6 Ma). *A. prolifera* is a known hybrid between the two. No microbial data are yet available for this species.

food uptake and contact with water and other organisms. For instance, Janelle Thompson at the Massachusetts Institute of Technology and her collaborators used 16S high-throughput tag sequencing to examine healthy and diseased coral morphospecies from different reefs. Although each coral has a unique core microbiome, it also carries a high proportion of non-specific visitors through contact with nearby surroundings.

Coral Holobiont Genomics

The microbial gene pool in a coral holobiont is orders of magnitude larger than that of the animal host, a fact with enormous implications for the physiology, ecology, and evolution of corals. Our ability to think of corals and their associated microbiota as single functional entities can help us to better understand how changing environmental conditions affect them. These



efforts will greatly benefit from genomics and postgenomics tools.

Although genomic data are not available for key members of the coral holobiont, this deficiency is being addressed. At least two coral genomes will be sequenced in the near future, as will *Symbiodinium* species from clades B and C. Meanwhile, the genomes of several coral pathogens are being sequenced, but many other associated microbial species are begging for attention. In contrast, the pioneering work on coral metagenomics by Forest Rohwer and colleagues at San Diego State University, has provided some early insight showing that microbial functional categories rapidly shift in coral reefs under environmental stress.

We continue to build our understanding of coral microbial assemblages and our knowledge that the health of coral ecosystems is a sensitive indicator of climate change and other environmental stresses. Because the overall health of

coral reef ecosystems depends on having a well-balanced microbial consortium, advancing our understanding of coral health and disease will surely improve our ability to implement effective coral-health management strategies.

Many analyses are under way, including profiling of host and algal transcriptomes, phylotyping and comparing microbial community compositions, genotyping coral hosts and *Symbiodinium* populations, and monitoring physiological and environmental data among naturally or experimentally disturbed coral holobionts. Such findings could guide efforts to incorporate microorganisms into corals that will enable them to respond to environmental change within ecological time frames. Ultimately, a systems biology approach that incorporates different “omics” in combination with theoretical but testable predictions will greatly enhance our understanding of coral reefs, which will prove critical in our efforts to protect these endangered ecosystems.

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