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Opinion

Genome Evolution of Coral Reef Symbionts as Intracellular Residents

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Coral reefs are sustained by symbioses between corals and symbiodiniacean dinoflagellates. These symbioses vary in the extent of their permanence in and specificity to the host. Although dinoflagellates are primarily free-living, Symbiodiniaceae diversified mainly as symbiotic lineages. Their genomes reveal conserved symbiosis-related gene functions and high sequence divergence. However, the evolutionary mechanisms that underpin the transition from the free-living lifestyle to symbiosis remain poorly understood. Here, we discuss the genome evolution of Symbiodiniaceae in diverse ecological niches across the broad spectrum of symbiotic associations, from free-living to putative obligate symbionts. We pose key questions regarding genome evolution *vis-à-vis* the transition of dinoflagellates from free-living to symbiotic and propose strategies for future research to better understand coral–dinoflagellate and other eukaryote–eukaryote symbioses.

Highlights

Coral reefs are sustained by long-term symbiosis between coral animals and dinoflagellate algae of the family Symbiodiniaceae.

Genomic studies have shed light on the molecular basis of the coral–dinoflagellate symbiosis.

Evolutionary mechanisms that underpin the transition of dinoflagellates in the family Symbiodiniaceae from free-living to symbiotic remain largely unknown.

Symbiodiniacean dinoflagellates are expected to share common evolutionary trajectories with other intracellular symbionts and parasites.

Comparison of the genome features of Symbiodiniaceae with those of other intracellular residents will improve our understanding of their evolutionary history and of other eukaryote–eukaryote symbioses.

Symbiodiniaceae: Critical Symbionts of Coral Reefs

Dinoflagellates of the family Symbiodiniaceae are the most prevalent photosynthetic symbionts in tropical and subtropical coral reef ecosystems. Although dinoflagellates are primarily free-living, Symbiodiniaceae have diversified mainly as symbiotic lineages. Previously classified within the genus *Symbiodinium* and colloquially known as zooxanthellae, Symbiodiniaceae are associated with diverse hosts including corals, jellyfish, clams, and foraminiferans. These symbiotic interactions can occur in the form of **mutualism** (see Glossary), **parasitism,** or **opportunism** and vary across a broad spectrum of associations determined by, for example, the permanence in the host, the host compartment in which the symbionts are found (intracellular or interstitial), host specificity, and transmission mode (**horizontal transmission**, **vertical transmission**, or both) [1–4]. The vast genetic and ecological diversity of these organisms prompted the recent systematic revision of Symbiodiniaceae into 15 (or more) clades with seven genera named [4]. Although dinoflagellate genomes are well known to be large (up to 250 Gbp in size) and complex [5], genomes of Symbiodiniaceae [1–1.5 giga base pairs (Gbp)] are relatively small [5,6]. Symbiodiniacean genomes therefore provide an excellent platform not only to analyze the evolution of the ubiquitous dinoflagellates but also to better understand the poorly understood evolutionary processes that underpin the establishment of eukaryote–eukaryote symbioses.

Intracellular residents (e.g., parasites, symbionts) undergo similar evolutionary trajectories, the stages of which include initial invasion into the host cell, permanence over generations in the intracellular environment, and transmission between hosts. Each of these stages impacts the evolution of resident genomes, leading to features that collectively constitute the so-called **resident genome syndrome** (Box 1) [7,8]. According to this notion, resident genomes pass through a highly dynamic and unstable phase characterized by extensive structural rearrangements during the initial transition to an intracellular lifestyle [9]. Confinement of the resident to the intracellular space then eventually leads to a more-stable, reduced genome [10,11]. In this stage, the

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Box 1. Evolutionary Implications of Intracellular Confinement

The resident genome syndrome posits a set of genome features (or symptoms) of intracellular residents that have arisen from long-term spatial confinement in the host cell. During the initial transition to confinement, the genomes of the residents are highly dynamic and unstable, with increased structural rearrangements and activity of mobile elements. Spatial confinement eventually leads to a reduced and more stable genome [9,21–23]. The reduced capacity of intracellular residents to undergo genetic recombination and the repeated bottlenecks they experience in transmission to other hosts result in a small N_e [7]. A small N_e hastens the fixation of newly emerging alleles (arising from mutation) regardless of their impact on the fitness of the residents; the subsequent accumulation of deleterious mutations is known as Muller's ratchet [52]. The underlying driver of Muller's ratchet, genetic drift [15], is reinforced by the relaxation of selective pressure on genes in the resident that encode functions redundant or neutral for the host [53].

Accumulation of deleterious mutations often results in genes with lost function (i.e., pseudogenes) that are prone to differential removal from the genome (i.e., deletion bias). Deletions can implicate one or a few bases or one or more genes. Together with the accumulation of substitutions in coding sequences, deletion biases lead to gene loss in resident genomes [54,55]. In diverse intracellular bacteria and some intracellular eukaryotes, genes encoding DNA repair functions are lost [21,43], contributing to the further degradation of their genomes. Accelerated mutation rate and reduced DNA repair capacity make underlying mutational biases evident. In coding regions of resident genomes, neutral accumulation of mutations is reflected in reduced preference of codons used [7].

Genome-size reduction can be driven by selective advantages of small genomes, deletion of mutated DNA, and/or a reduced chance of incorporating exogenous DNA [7,54,55]. Potential selective advantages of smaller genomes include reduced metabolic costs for the maintenance and replication of DNA and faster DNA replication (and thus shorter life cycles) [7,56]. LGT, the major force counteracting genome reduction in bacteria, is limited in the restrictive intracellular environment [8,54]. However, host cells infected with multiple residents simultaneously open the possibility of extending the gene inventories of these residents. This proposed 'intracellular arena' hypothesis [57] was supported by previous studies of *Wolbachia* endosymbionts [58] and microsporidian parasites [59].

genomes are generally small and A+T rich and the genes display high evolutionary rates. These symptoms have largely been described in the genomes of intracellular bacteria [7,8].

Currently available genome data from Symbiodiniaceae reveal signatures of symbiosis-related gene functions [12–14] but the impact on these genomes of the evolutionary transition to intracellularity remains little explored. Here, we discuss the genome evolution of Symbiodiniaceae across the broad spectrum of symbiotic associations (Figure 1) focusing on the connecting scenarios of free-living species, facultative symbionts, and obligate symbionts.

Free-Living Species

At one end of the spectrum, some Symbiodiniaceae species have not been found to be associated with a host. These free-living taxa include some species in the *Symbiodinium* genus (former Clade A; e.g., the type species *S. natans* and *S. pilosum*), the exclusively free-living *Effrenium* genus (former Clade E), and *Fugacium* (former Clade F) [2,4].

A free-living lifestyle presents opportunities for the exchange of genetic material (e.g., recombination via sexual reproduction) with conspecifics, facilitates **lateral genetic transfer (LGT)** due to exposure to other organisms, and avoids the bottlenecks of transmission between hosts, all of which counteract the effects of confinement to the intracellular space [9,15] (Figure 1A). Fluctuating environmental conditions and the access to different habitat types often require a broader gene repertoire and increased selective pressure for maintaining a range of metabolic functions [8]. A recent study demonstrates that conserved lineage-specific genes of unknown function in dinoflagellates might play a role in niche specialization [16]. We expect these features to be common in the genomes of free-living Symbiodiniaceae as well as in other dinoflagellates.

Although the genome sequence of the free-living *Fugacium kawagutii* [12,14] is available, the rates of recombination and LGT have not been systematically assessed. High genetic diversity and a near-complete meiotic gene set have been reported in symbiodiniacean genomes [14,17],

Glossary

Effective population size (N_e): in population genetics, the number of effectively reproducing individuals under the assumption of an ideal population.
Genetic drift: evolutionary mechanism in which the changes in allele frequencies of a population are driven by chance.
Genome phasing: statistical estimation of alleles (or haplotypes) from potentially heterozygous genome data.
Horizontal transmission: mode of symbiont transmission in corals in which the dinoflagellate symbionts can be acquired from the environment.
Intracellular resident: any unicellular organism that has adapted to a lifestyle inside the cell(s) of another organism (host).
Lateral genetic transfer (LGT): uptake and establishment of genetic material from one organism to another, instead of vertical parent-to-offspring inheritance.
Muller's ratchet: accumulation of nonreversible deleterious mutations in a population as a consequence of asexual reproduction (or lack of recombination); named after the American geneticist Hermann Joseph Muller.
Mutualism: type of symbiotic association in which the symbiont lives on or in another organism (the host) such that both the symbiont and the host benefit from interacting with each other.
Opportunism: type of symbiotic association in which the symbiont (opportunist) lives on or in another organism (the host) that is experiencing detrimental conditions.
Optical mapping: technique in which mapped locations of restriction enzyme sites in a genome are used to construct ordered maps to facilitate the determination of distances between and the ordering of DNA fragments in a genome assembly.
Parasitism: type of symbiotic association in which the symbiont (parasite) lives on or in another organism (the host) causing the host some loss of fitness.
Resident genome syndrome: series of genome features shared by intracellular residents, the most remarkable being genome reduction, accelerated evolutionary rates, and mutation bias; originally described for bacteria [7,8].

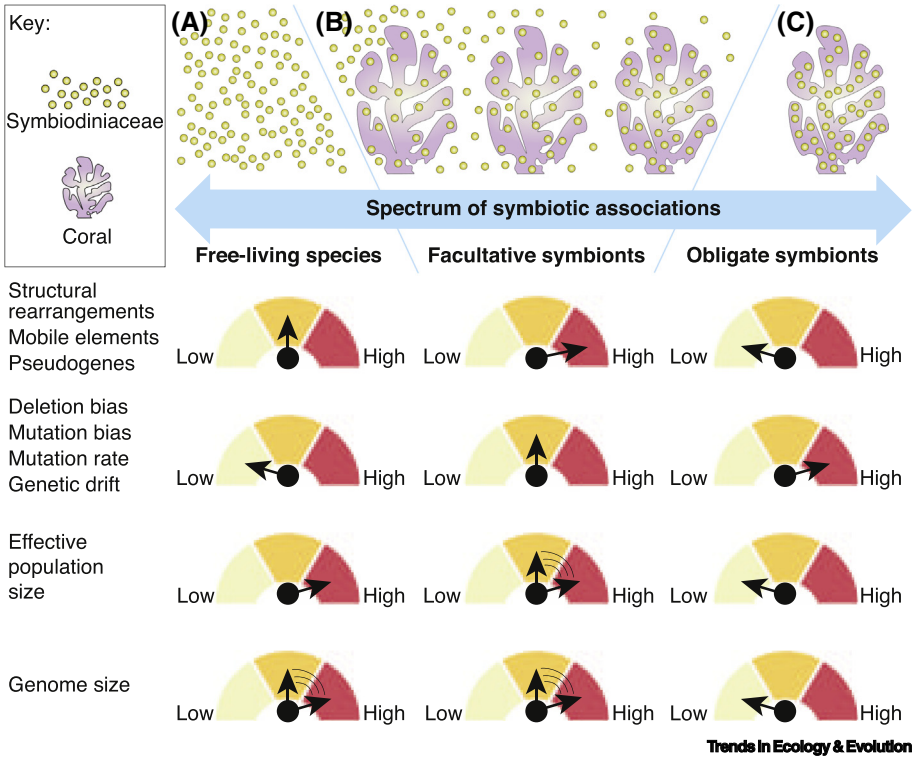

Figure 1. Expected Genome Features of Symbiodiniaceae across the Broad Spectrum of Symbiotic Associations. Expected genome features of coral reef symbionts across the spectrum connecting the scenarios of (A) free-living species, (B) facultative symbionts, and (C) obligate symbionts. The meter arrows show the level (or amount) of a set of genome features expected to be seen in a scenario relative to the others.

Synteny: co-occurrence of genetic elements in a specified region of a genome.
Vertical transmission: mode of symbiont transmission in corals in which the dinoflagellate symbionts are passed on through coral generations from parents to offspring.

suggesting the capacity for sexual reproduction [18]. However, direct observations of sexual reproduction in these taxa have not been possible; thus, the recombination rate cannot be determined. Likewise, assessing LGT in symbiodiniacean genomes is challenging because of the acquisition of exogenous genes from multiple endosymbiotic events involving prokaryote and eukaryote sources, as demonstrated by the complex history of plastid origin in dinoflagellates [19,20]. Fragmented genome assemblies derived from short-read sequence data limit our capacity to corroborate the origin of sequences sharing substantial similarity with bacterial and viral genomes, which have thus far largely been regarded as contaminants [13,14]. This challenge can be overcome by incorporating long-read sequence data in genome assembly.

Genomes from other species in the Order Suessiales, including the free-living *Polarella glacialis* and the symbiotic *Pelagodinium béii*, represent important outgroup references to understand the evolutionary transition from a free-living to a symbiotic lifestyle and the origin of Symbiodiniaceae. Comparative genomics of organisms in these taxa will reveal structural (e.g., shared **synteny** and interspersed repeat landscapes) and functional features (e.g., gene content, gene duplication, metabolic pathways) that are unique to Symbiodiniaceae.

Facultative Symbionts

Most Symbiodiniaceae are symbiotic, representing a broad spectrum of symbiotic associations and a range of host specificity. Their genomes would have experienced the phase of genome instability during the early transition stages to an intracellular lifestyle (and symbiosis). The genomes

during this phase may be larger than those of well-established residents and have accumulated extensive structural rearrangements, mobile elements, and pseudogenes (Figure 1B), as observed in other facultative and recently established residents [9,21–24].

As facultative symbionts, *ex hospite* stages (cells outside a host) are common in the life cycle of these species. Corals are known to adjust symbiont density regularly by expelling Symbiodiniaceae to the external environment [25]. On expulsion, the viable symbiodiniacean cells may reproduce sexually with conspecifics *ex hospite* in a cell-dense environment, boosting the recombination rate that may be even higher than that of their free-living relatives. However, the low viability of these *ex hospite* cells [26] argues against this notion. These competing hypotheses remain to be systematically tested.

Cladocopium goreaui (formerly *Symbiodinium goreaui*, or type C1) is a host generalist. Reported from >150 coral species in Australia's Great Barrier Reef [27], *C. goreaui* is largely horizontally transmitted. *C. goreaui* shows the highest level of genome-fragment duplication (implicating ~15.3% of its genes) compared with *Symbiodinium microadriaticum*, *Breviolum minutum*, and *Fugacium kawagutii* (implicating <6% of the genes of each) [12–14]. *S. microadriaticum* and *B. minutum* generally exhibit a narrower host range [28,29] than *C. goreaui*, while *F. kawagutii* is free-living [4].

Only modest synteny is shared among symbiodiniacean genomes of different genera, suggesting a high extent of structural rearrangements [12–14]. Some structural rearrangements can be attributed to the activity of transposable elements [12]. A recent study [14] revealed an ancient burst of mobile elements in the genomes of all four analyzed species of distinct symbiodiniacean genera, including a member of the most-basal lineage *Symbiodinium* (formerly Clade A), *S. microadriaticum*. These data, albeit limited, indicate that the burst of mobile element activity is likely to have predated the radiation of Symbiodiniaceae and may be associated with the early evolutionary transition to intracellularity of these lineages. However, most interspersed repetitive elements in these genomes remain uncharacterized. Conservation of these elements within the symbiodiniacean lineages may elucidate their roles in the transition to intracellularity.

Whether these observed features are a consequence of a facultative symbiotic lifestyle in Symbiodiniaceae remains an open question. All published genome assemblies [12–14,30,31], derived mostly from short-read sequence data, are highly fragmented. The implementation of emerging genomic technologies across isolates from the same genus (e.g., *Cladocopium* spp.) will enable researchers to address this question more effectively. Specifically, long-read (~20–50 kbp) sequence data can span larger indels and resolve repetitive genomic regions more effectively than short-read data (typically 100–150 bp in length). In addition, duplication and translocation of genome regions can be better characterized by **optical mapping** of long (>250 kbp) DNA molecules and by **genome phasing** [32,33].

Obligate Symbionts

At the other end of the spectrum, some Symbiodiniaceae may be obligate symbionts. These taxa are rarely, if at all, found in the environment or reported in culture. However, one cannot dismiss that brief *ex hospite* stages may still occur due to regular adjustments of symbiont density by the hosts. In the scenario of strict obligate symbionts (Figure 1C), genomes are expected to follow the evolutionary trajectory postulated in the resident genome syndrome (Box 1).

The **effective population size (N_e)** measures the impact of **genetic drift** on the evolution of a lineage. Despite the existence of a theoretical framework in population genetics for vertically transmitted symbionts [34] and access to a range of methods to estimate N_e from genetic data

[35] (F.C. Wham, Thesis dissertation, Pennsylvania State University, 2015), estimates of N_e for populations of Symbiodiniaceae are currently lacking. Nonetheless, small N_e (together with low gene flow, dominance of clonal reproduction, and local adaptation) has been inferred to result in genetic differentiation among symbiont populations inhabiting the same coral species in different reef locations [26]. Population studies can also contribute to our understanding (*inter alia*) of the rate of sexual versus asexual reproduction in Symbiodiniaceae, the adaptation potential of reefs based on the diversity of symbiont genotypes available both inside and outside the coral hosts and the functional role of those genotypes, the differences in evolutionary patterns between different types (e.g., host specialists versus host generalists), and the delimitation of cryptic species [26,36,37]. We look forward to future population-genetic/genomic studies of different symbiodiniacean ecotypes, particularly in exploring bottleneck effects during the between-host transmission on population dynamics, similar to earlier studies of prokaryotic residents [38,39].

Muller's ratchet can lead to loss of phylogenetic signal as homologous sequences become more dissimilar. In microsporidia, a specialized group of intracellular parasitic fungi, accelerated mutation rate in protein-coding sequences is uncoupled from genome architecture [11] and complicates their positioning in phylogenetic trees [40]. In light of the high sequence divergence within Symbiodiniaceae [41], these lineages should be scrutinized further to identify pseudogenes and other factors potentially contributing to the extensive differences observed in gene families [42].

Across a wide range of intracellular bacteria and some intracellular eukaryotes, genes encoding functions in DNA repair are lost [21,43], contributing to the further degradation of their genomes. Conversely, functions associated with nucleotide-excision DNA repair are enriched in the core genes of Symbiodiniaceae. This observation may reflect adaptation of Symbiodiniaceae to high UV environments [42] and reveal a mechanism that counteracts genome reduction caused by spatial confinement.

Mutation bias (towards high A+T) is well known in the genomes of intracellular bacteria [44,45] but is less evident in intracellular eukaryotes. Nonetheless, the highest A+T and G+C contents in eukaryote genomes (to our knowledge) are observed in *Plasmodium falciparum* and *Chlorella variabilis* NC64A, respectively; both are known to occur as intracellular residents [24,46]. Base composition also varies substantially among different regions of these genomes. In the *C. variabilis* genome, the regions with the lowest G+C content are repeat poor and contain genes with shorter introns and exons, and lower codon-usage bias; G+C content in this green alga also correlates with gene expression and intron size [24]. In the nucleomorph genome of a cryptomonad (*Guillardia theta*) [47], G+C content varies from 46% in terminal repeats to 35% in tRNAs and plastid genes, and 23% in housekeeping genes. These examples suggest that the impact of confinement to the intracellular space on genome evolution of eukaryotes also varies from one type of genomic region to another, depending in part on the potential of these regions to vary in base composition while maintaining function. Symbiodiniacean genomes do not display substantial mutational biases; rather, their G+C content resembles that of other dinoflagellates [5] both globally (between 43.0% and 51.5%) and in the protein-coding regions (between 50.4% and 58.6%). Reduced codon-usage preferences in symbiodiniacean genomes compared with other dinoflagellates may reflect genetic drift acting on coding sequences, although a higher G+C content in the third codon positions opposes this notion [42,48]. In addition, smaller genome sizes in Symbiodiniaceae relative to those of other dinoflagellates are probably not solely due to their endosymbiotic lifestyle, because the trend of genome reduction is observed in earlier-diverging free-living lineages (Figure 2) [6,49].

Cladocopium type C15 cells are highly host specific, found only in the ubiquitous stony corals of *Porites* spp. These symbionts did not survive in media simulating the marine environment and

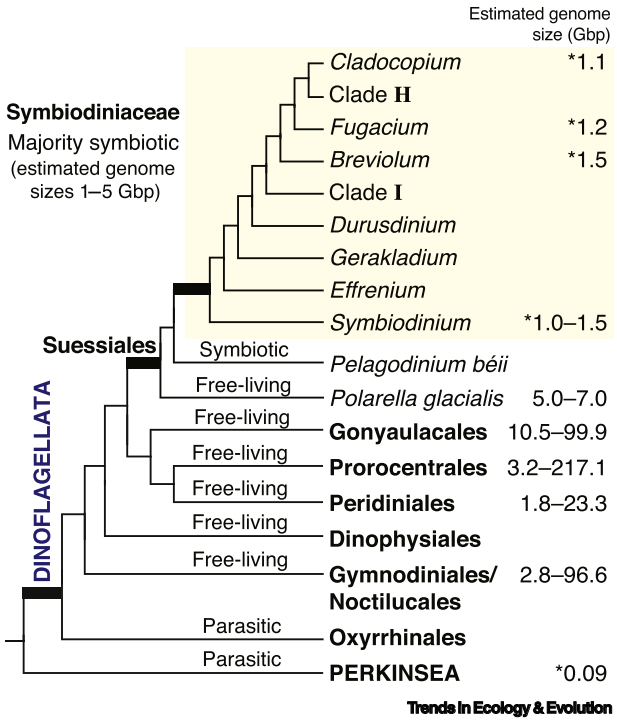

Figure 2. Estimated Genome Sizes of Dinoflagellates. Estimated genome sizes of dinoflagellates, shown on the dinoflagellate phylogeny [16]. Genome sizes estimated based on sequencing data are asterisked (*); all other estimates are based on 4′,6-diamidino-2-phenylindole (DAPI) fluorescence staining [6]. Abbreviation: Gbp, giga base pairs.

Outstanding Questions

How often do Symbiodiniaceae reproduce sexually and under what conditions?

How viable are *ex hospite* cells in distinct symbiotic Symbiodiniaceae taxa?

Does the burst of mobile element activity in symbiodiniacean genomes relate to the transition to symbiosis and/or the diversification of these lineages?

To what extent do genomes of symbiotic Symbiodiniaceae and other eukaryote residents exhibit symptoms of the resident genome syndrome?

Can the accelerated evolutionary rates typical of intracellular residents explain the extensive genome-sequence divergence among symbiodiniacean lineages?

Can the spectrum of symbiotic associations in Symbiodiniaceae be explained by intrinsic molecular mechanisms that counteract genome reduction?

What drove genome-size reduction in dinoflagellates of the order Suessiales?

their survival in media emulating the *in hospite* conditions of the host was limited to a few days [50]. For these reasons, C15 cells resemble obligate symbionts, and their genomes remain largely inaccessible. New approaches to extract genome data from symbionts *in hospite* (e.g., isolation of symbiodiniacean DNA from coral tissue) are thus needed. Genomes of these *bona fide* symbionts represent a critical reference for comparison against genomes of other symbiotic and free-living species. By integrating genome data from the symbionts with those from the associated coral host and the microbiome in a hologenome analysis, one can also delineate the contribution of each biotic component (at the molecular level) to sustaining a healthy coral holobiont.

Concluding Remarks and Future Perspectives

The recent availability of draft genomes from Symbiodiniaceae and systematic revision of these taxa are allowing investigators to venture deeper into the evolutionary history of these ecologically important organisms. In this Opinion article, we discuss the genome evolution of Symbiodiniaceae across the broad spectrum of symbiotic associations. We acknowledge that Symbiodiniaceae ecology is highly intricate. For instance, host specificity does not always correlate with transmission mode or with the facultativeness of symbiotic association. It is also likely that the lifestyle of extant taxa has changed multiple times over the long evolutionary history of this lineage. However, we believe that these scenarios pose useful models for assessing the evolution of symbiosis in Symbiodiniaceae. Research efforts should leverage and integrate current knowledge of Symbiodiniaceae with that of other symbiotic organisms that share similar evolutionary trajectories to answer fundamental but open questions about the evolution of this group (see Outstanding Questions). Evidently, the biology of Symbiodiniaceae deviates from that of other models in this research area (e.g., bacterial symbionts, parasites), but these deviations represent areas of opportunity for research.

We foresee the comparative genomics of diverse lineages becoming more specialized, using genome data from more species in each of the newly established genera, to address biological questions that are more narrowly focused. Such questions would include which gene functions contribute to heat tolerance in *Durusdinium* spp. (former Clade D), the hyperdiversity of *Cladocopium* spp. (former Clade C), or the core genome features (and gene functions) in the most basal lineage of Symbiodiniaceae, *Symbiodinium*. Population-scale genomic analysis represents a powerful tool to assess genetic diversity in distinct populations and for each species. It will also become possible to investigate the deep evolutionary divergence of symbiodiniacean lineages, probing the ancestral characteristics that gave rise to these remarkably diverse and ecologically important lineages. Certainly, this research will increasingly rely on other widely scoped surveys beyond traditional comparative genomics and transcriptomics, to incorporate proteomics, metabolomics, epigenomics, post-transcriptional regulation (e.g., by small RNAs), and their associated experimental validation; concerted, multidisciplinary collaborations will become a norm. Finally, extending beyond Symbiodiniaceae, and in combination with genome-scale data from the associated hosts and microbiome, hologenomics [51] represents a promising approach to gain a comprehensive snapshot of various symbiotic associations that are critical to coral reefs.

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