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Resolving structure and function of metaorganisms through a holistic framework combining reductionist and integrative approaches

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Abstract

Current research highlights the importance of associated microbes in contributing to the functioning, health, and even adaptation of their animal, plant, and fungal hosts. As such, we are witnessing a shift in research that moves away from focusing on the eukaryotic host *sensu stricto* to research into the complex conglomerate of the host and its associated microorganisms (i.e., microbial eukaryotes, archaea, bacteria, and viruses), the so-called metaorganism, as the biological entity. While recent research supports and encourages the adoption of such an integrative view, it must be understood that microorganisms are not involved in all host processes and not all associated microorganisms are functionally important. As such, our intention here is to provide a critical review and evaluation of perspectives and limitations relevant to studying organisms in a metaorganism framework and the functional toolbox available to do so. We note that marker gene-guided approaches that primarily characterize microbial diversity are a first step in delineating associated microbes but are not sufficient to establish proof of their functional relevance. More sophisticated tools and experiments are necessary to reveal the specific functions of associated microbes. This can be accomplished through the study of metaorganisms in less complex environments, the targeted manipulation of microbial associates, or work at the mechanistic level with the toolbox available in model systems. We conclude that the metaorganism framework is a powerful new concept to help provide answers to longstanding biological questions such as the evolution and ecology of organismal complexity and the importance of organismal symbioses to ecosystem functioning. The intricacy of the metaorganism requires a holistic framework combining reductionist and integrative approaches to resolve metaorganism identities and to disclose the various roles that microorganisms play in the biology of their hosts.

1. Introduction

Recent years have brought a changing imperative in the life sciences, sparked by the revolution of genomic tools for studying the molecular nature of organisms (McFall-Ngai et al., 2013; Bordenstein and Theis, 2015; Bang et al., 2018). Contrary to the classical view that microbes are primarily pathogenic and disease-causing, there is now a multitude of studies indicating that a host-specific microbiome provides functions related to the metabolism, immunity, and environmental adaptation of their animal, plant, and fungal hosts (Fraune et al., 2015; Moran and Yun, 2015; Roder et al., 2015; Hume et al., 2016; Mortzfeld et al., 2016; Röthig et al., 2016; Araldi-Brondolo et al., 2017; Ochsenkühn et al., 2017; Shaffer et al., 2017; Ziegler et al., 2017). Similarly, microbes have been found to be important for environmental sensing (Unabia and Hadfield, 1999), inducing sexual reproduction in choanoflagellates (Woznica et al., 2017), and contributing to developmental transitions (Leitz and Wagner, 1993; Webster et al., 2004). More recently, Rook et al. (2017) proposed that life history traits such as developmental pace and longevity are in part determined by the organism's microbial associations. It is becoming increasingly clear that animals, plants, and fungi evolved within a microbial world and that such multicellular organisms rely on their associated microbes for many aspects of their function, especially with regard to living in extreme environments such as deserts, oligotrophic seas, or hydrothermal vents (Bang et al., 2018). However, even though a broader appreciation of the importance of microbes has emerged, we still know comparatively little about the different niche spaces (compartments) that multicellular hosts provide, how such niches determine microbiome composition and function, and how the often-complex assemblages of microbes interact with one another and their hosts in a mechanistic sense.

2. The metaorganism concept and the challenges of metaorganism research

To address such questions, scientists from diverse disciplines have converged on exploring microbiomes associated with host organisms using a new conceptual framework – the metaorganism. The popularity of the metaorganism framework has led to a proliferation of terms to describe the sum of the multicellular host and its associated microorganisms (see Table 1). While the terms "metaorganism" and "holobiont" generally have been used interchangeably, we propose that these terms be used to distinguish different kinds of microbial associations. The term metaorganism is used herein to refer to the host organism and those components of its associated microbiome to which function has been either ascribed or for which there are reasonable grounds to suspect it; in contrast, the term holobiont is used in the more traditional context of the entire diversity associated with a host organism (Table 1). In this context it is important to note that, given the diverse disciplines studying metaorganisms, "function" is not defined strictly and often is used with different meanings. In an evolutionary sense, for instance, any assigned microbial function may imply a fitness effect on the host. In an ecological context, it may refer to the function of a microbe in the context of the metaorganism and its role in the ecosystem, whereas in a genomic context, function may refer to an actual expressed gene product or protein. Here, we refer to microbial function in the broad context of a contribution (beneficial or detrimental) to the metaorganism. Importantly, even when a microbial contribution to the host organism can be defined and is used to define the metaorganism concept, a metaorganism (like a holobiont) is specific to a time and place and not static. As such, we must acknowledge an uncertainty with regard to our ability to identify all functionally relevant microbes given the temporal ('fluidic') nature of host-microbial interactions, as well as the possibility of competitive exclusion of detrimental microbes by other associated microbes. To maintain clarity the term "metaorganism" (or holobiont) should not be confused with what sociobiologists call a *superorganism* – a

term coined to describe the communities of social insects, such as leaf cutter ants or termites, and their associated structures (Hölldobler and Wilson, 2009), which should not be used in the current context.

More broadly, the host-associated microbiome is central to the holobiont and metaorganism concept. The microbiome consists of microorganisms or microbes such as bacteria, archaea, protists, and fungi (while acknowledging the fact that some of these organisms, such as protists and fungi, themselves can be considered hosts of other microorganisms), but also viruses (Grasis, 2017). As an example, the *Hydra* metaorganism is composed of the animal host, a suite of bacteria, and associated viruses (Bosch and Miller, 2016). In comparison, a coral metaorganism is not only composed of the animal host, a suite of bacteria, fungi, and viruses (Knowlton and Rohwer, 2003; Bang et al., 2018), but also obligate intracellular algal symbionts of the family Symbiodiniaceae (LaJeunesse et al., 2018).

Coral metaorganisms in particular highlight the importance of microbes to host function since they enable their animal hosts to live in otherwise inhospitable environments (Muscatine and Porter, 1977; Bang et al., 2018): export of photosynthates from micro-algal endosymbionts can provide up to 95% of the energy requirements of the host coral. This allows corals to build – in oligotrophic seas – massive calcium carbonate skeletons, the three-dimensional structures that form the foundations of reef ecosystems. Thus, the symbiosis between Symbiodiniaceae and their coral hosts allows them to become the engineers of entire ecosystems (Jones et al., 1994). In this way they serve in a similar role to plant metaorganisms in terrestrial environments, where the capacity of root symbionts to improve the uptake of nutrients and water, or of foliar symbionts to regulate ingestion by herbivores and pathogen infection, reflects how microbes can change the capacity of hosts to colonize and flourish in the context of biotic and abiotic challenges, thus shaping terrestrial, aquatic, and marine systems (Arnold et al., 2003; Friesen et al., 2011; Ortiz et al., 2015; Pérez-Jaramillo et al., 2018; Fitzpatrick et al., 2018). Curiously enough however, the 'holobiont' or 'metaorganism' terminology entered the botanical lexicon only recently (e.g., Cregger et al., 2018). Together, these studies show that interactions between microbiomes and their hosts are ecologically and evolutionarily powerful across the tree of life.

While in specific cases (such as the examples cited above) the evidence for the importance of the associated microorganisms is compelling and the definition of a metaorganism might seem straightforward, in the real world it is often much less clear which microorganisms are functionally important. The example of the coral–Symbiodiniaceae partnership is obvious, in part because the algae live inside the cells of their animal host, but for the majority of study systems the delimitation is not clearly defined. For example, microbes associated with external surfaces could either interact functionally with their hosts or their presence could simply be accidental. Associations can be transitory or long-term, with little evidence that the duration of affiliation – especially for horizontally transmitted taxa – can be taken as a proxy for functionality (even just temporarily "associated" microbes can be functionally consequential, as is the case for pathogens). Also, microbes found on or within a host may be commensal with no clear function, or their functional impacts may emerge only under particular stresses rarely encountered in the laboratory or *in vitro*. For these reasons, there is an ongoing debate on how to discern these more or less integrated associations of organisms, and where to categorize phenomena that are fundamentally gradational (Moran and Sloan, 2015; Queller and Strassmann, 2016; Skillings, 2016; Doolittle and Inkpen, 2018; Rosenberg and Zilber-Rosenberg, 2018). Host niche space, i.e. physical containment, offers an obvious first-order limit, hence the focus on multicellular animal and plant hosts along with their contained microbiomes. Such consortia are distributed pervasively across the tree of life: cellular endosymbionts, gut microbiomes, endophytic fungi, nodule-contained rhizobia, and endohyphal bacteria are all relatively easy to define in

such terms, though even here the definition can be blurred when such organisms have extracellular/extra-host phases in their life cycle or can exist in some form outside the host body. More often than not, however, the challenge is more fundamental: to understand the functional roles of microbes and to define the metaorganism in a tractable and mechanistic way. We will discuss such criteria in more detail in the following, alongside an examination of the circumstances under which the metaorganism concept is useful and of the tools one should employ to study functional aspects of host–microbe associations.

Table 1: Terminology

Term	Definition
Model organism	A species that has a range of characteristics that are particularly advantageous for studying a particular biological trait. For example, *Aplysia californica* is a well-studied model organism in neurobiology and neuroscience, due to its unusually large neurons (caused by polyploidy). Primary criteria for the selection of model organisms used to be ease of maintenance and experimental manipulation, but now often also include factors such as genome size and genetic tractability.
Non-model organism	Organisms that are not as widely studied and for which only a limited set of resources might be available. They may lack the features that make model organisms easy to investigate (e.g. they can be hard or expensive to grow in the laboratory, or may have long life cycles, low fecundity, or poor genetic tractability). In some cases, they simply do not have the long history of study that has provided the foundation for the choice of model organisms in certain disciplines. As model organisms represent only a very limited scope of the diversity and function in nature, the study of non-model organisms is relevant and important for understanding the possible inferences and limitations of model system studies and the ways in which model organisms can be used to interpret the ecology of species and their role in ecosystems.
Model system	A representative species for a particular discipline, but less popular, generalized, or developed than a model organism. For instance, the sea anemone *Aiptasia* is a model system for the coral–algal symbiosis that forms the basis of coral reef ecosystems.
Microbiome	The sum of microbes in a particular environment, organism, or part of an organism (e.g., the gut, the epidermis, the leaf). Commonly the term also refers to the entire collection of genes of all the microbes in a community.
Holobiont	The eukaryotic host with all external and internal associates. This multispecies consortium can include bacteria, archaea, protists, fungi, and viruses. All associated member species are considered, regardless of their being transient or permanent or whether they form a functional association with the host or other microbes or not.
Metaorganism	A metaorganism is the sum of a eukaryotic host and its associated species in a narrower context as compared to the holobiont, with the focus on those associates for which function, i.e. any form of contribution (beneficial or detrimental) to the metaorganism, is known or implied. The term metaorganism therefore has implications for the function of a holobiont in a given environment. The functional aspect depends on the identity, activity, and abundance of the associated partners. Likewise, whether a specific function/microbe is functionally relevant can depend on host developmental stage, age, reproductive state, or physiological condition.

	As such, a metaorganism (like a holobiont) is specific to a time and place, and not static.
Hologenome	The collective genomic content or genetic information encoded by the eukaryotic host and all the species associated with it. The hologenome concept often carries the controversial assumption that, to a significant extent, selection acts at the holobiont level, a view that is hotly debated.

3. Perspectives and limitations of studying organisms in a metaorganism framework

In a simple sense, metaorganisms may function as closely integrated ecosystems – specialized environments with community members that have direct and indirect impacts on one another. Such close coupling can make the exchange of information and materials more direct, rapid, and secure than in the outside world. As such, multicellular hosts can be thought of as modular systems, containing microbes of different types and properties and unique opportunities for material and informational exchange. Within ecosystems, certain roles or ecosystem functions can be provided by different members of the community. Similarly, in host organisms certain functions can be performed by different microbes that have converged mechanistically or otherwise to provide the same "ecosystem service", as proposed by the "it's the song not the singer" concept (Doolittle and Inkpen, 2018). However, unequivocal experimental evidence for widespread functional redundancy is missing to date in holobiont/metaorganism systems. Nevertheless, evolutionary theory highlights the importance of function rather than species identity *per se* – a concept also presented by the trait-based approach with regard to understanding ecosystem assemblage and function (Kiørboe et al., 2018). This framework argues for a functional understanding of microbiomes rather than one based on lineages or microbial identity alone.

Reflections on the functional aspects of microbiomes in the metaorganism often return to a central point – the evolutionary origins of such assemblages and their roles. Multicellularity arose relatively late during the history of Earth, emerging in a microbial world and providing novel substrates and interactions for diverse microbial lineages. Although difficult to reconstruct, the evolutionary origin of metaorganisms might be linked to a beneficial sub-contracted division of labor, potentially starting out from commensal coincidences that over time changed into mutually beneficial relationships. Once established, these novel relationships offered various advantages, such as free and reliable food delivery, protection from environmental stress, containment for collective digestion, infrastructure for large-scale transport, or a new apparatus for gas exchange. Such intimate cohabitation would then lead to the emergence of a metaorganism grade of organization as various metabolic tasks are taken over by associated microbes, often due to the superiority of microorganisms in metabolizing many kinds of substrates or their capacity for rapid adaptation via their extremely large population sizes or by means of horizontal gene transfer (Theis et al., 2016).

But it would be wrong to assume that just because associated organisms often play an important role in connection with their hosts, that this role is fundamental to the host's existence: current usage doesn't necessarily reflect evolutionary origins or essential association. Rather, in a world permeated by microbes, all organisms will by default be associated with microbes, and some of these may take on functions previously fulfilled by the host. However, the capacity to rapidly acquire novel functions may be central to the evolutionary history of metaorganisms, and indeed those we see today are the 'success stories' that may disproportionately bias us to think of microbial symbioses as central to host success. Instead, such associations may represent one of several potential optima, and certainly one of great impact – but not the

only solution, and not always as central to success as might be anticipated. Indeed, in nutrient-replete situations mycorrhizal fungi can shift to playing a parasitic role, gaining more than they give (Schmidt et al., 2011).

Yet it is unquestionable that microbes can be central to the origin of evolutionary innovations in multicellular hosts. For instance, it was recently proposed that nervous systems evolved as much to control associated microbes as to manage sensory inputs and muscle control (Klimovich and Bosch, 2018). Likewise, innate immunity in invertebrates evolved not only to fight off detrimental microbes, but also to recognize beneficial ones (Bosch, 2014; Rook et al., 2017). Challenging as it may be, we need to define experiments and tools that can be used to disentangle such complex relationships and dependencies in order to be able to differentiate between cause and consequence, and causation and correlation. At present, diverse tools have been developed for the purpose of inferring function, and they increasingly complement marker gene sequencing that defined the first phases of microbiome studies in host organisms and other environments (Fig. 1).

4. Experimental design considerations and functional tools

4.1. Marker gene approaches and their limitations

What has led to the newly discovered importance of bacteria is our novel ability to sequence marker genes and thus to estimate microbial diversity at an unprecedented depth and at decreasing costs, due to the advent of next-generation sequencing (NGS) (Tringe and Hugenholtz, 2008). The use of 16S rRNA gene sequencing for archaea and bacteria, alongside various other methods to describe eukaryotic microbial diversity, has ushered in a new era of microbial identification without the limitations of culture-based approaches. Before that, the description of bacteria associated with organisms and environments was expensive, characterized by low throughput, and relied on labor-intensive cloning-and-sequencing approaches. NGS approaches have revolutionized our understanding of microbial diversity and microbe distribution across the local and global scales (Tedersoo et al., 2014; Davison et al., 2015; Delgado-Baquerizo et al., 2018). The contemporary perspective is that bacteria (and often, other microbes as well) can be found in all environments, even under conditions previously thought to be inhospitable, e.g., at temperatures exceeding 80 °C (Stetter, 1996), at extreme salinity or at high concentrations of heavy metals (Antunes et al., 2011). Along with this comes the notion that bacterial diversity is nearly inexhaustible; indeed, a recent study estimated the total number of distinct microbial taxa to be approximately 1 trillion (10^{12}) (Locey and Lennon, 2016). The problem is that characterizing microbial diversity using marker gene approaches is straightforward enough, but that this kind of data is of limited value in terms of understanding function; microbial taxa may turn out to be phylogenetically different although they have the same function, or they may be phylogenetically similar but serve very different functions (Burke et al., 2011).

Therefore, metagenome and metatranscriptome analyses are required in order to detect differences in the presence of enzymes and pathways. Incorporating all genes and proteins allows to infer functional redundancies and to inform functional redundancy vs. phylogenetic difference. Similarly, describing microbial diversity using marker gene surveys provides no information on the location or association of the respective bacteria within or on the host. As such, visualization of microbes in or on host organisms via FISH, FISH-CLEM, CARD-FISH, SEM techniques, or *in vivo* labeling with fluorescent proteins (Hannig et al., 2010; Neave et al., 2016; Araldi-Brondolo et al., 2017; Wein et al., 2018) can supplement metagenome/metatranscriptome data in establishing function. Visual investigation further allows for

approximating microbial density ('carrying capacity'), which in itself can be an indication of the relative importance of microbes. Estimates of carrying capacities can be rechecked by using targeted approaches such as FACS, quantitative PCR, or counts of colony forming units (CFUs) (Wein et al., 2018). Further evidence for a functional relationship might come from studying the metabolite exchange between microbes and their hosts, e.g. via ToF- and NanoSIMS approaches that provide a currently unmatched methodology for imaging and measuring the exchange of defined metabolites at the single-cell level (Rädecker et al., 2018; Raina et al., 2018). These approaches are facilitated by the availability of cultured isolates that allow for detailed characterization and manipulation, e.g., with reference genome sequencing (Neave et al., 2014), elucidation of growth conditions, *ex situ* incubations (Cardenas et al., 2018), and targeted functional activity testing (e.g., quorum sensing, quorum quenching) (Pietschke et al., 2017). Lastly, the ability to conduct experiments with organisms that are largely (gnotobiotic) or completely (axenic) devoid of microbes allows for detailed insights into the contributions of microbes to metaorganism function (Fraune et al., 2015; Domin et al., 2018). In particular, the possibility of combining gnotobiotic animals and cultured microbial isolates allows for re-colonization experiments that help to unequivocally assign functions to specific microbes (Voolstra, 2013; Fraune et al., 2015; Domin et al., 2018) as well as to determine the colonization dynamics of microbes (Domin et al., 2018; Wein et al., 2018), although bacteria–bacteria interactions also need to be considered (Fraune et al., 2015; Li et al., 2015).

Despite the above considerations, marker gene sequencing approaches are currently *en vogue* due to their ease and feasibility. They represent an imperfect but legitimate approach to characterizing microbial diversity and community composition, but the step to inferring function remains to be taken. As such, 16S-based surveys should only be considered the first step of many on the way to gaining a more comprehensive understanding of the relationship between hosts and their associated microbes (Fig. 1).

4.2. The importance of less complex environments and model systems

The suite of approaches available for investigating function in bacteria–host associations (Fig. 1) highlights the need for collaboration – the scale and breadth of such efforts means that they often are beyond the scope of individual laboratories. One corollary of this is that real progress requires that researchers agree at some level to focus on a limited set of organisms for which a range of such methods are available. The selection of appropriate model systems should consider the complexity of the microbiome as well as the ability to manipulate key associated microbes. In addition, there is a growing recognition of the importance of non-bilaterian host organisms in metaorganism research. For instance, early-diverging metazoans such as *Hydra*, *Nematostella*, and *Aiptasia* are all cnidarians and sister group to bilaterians. Consequently, their phylogenetic position makes them ideal candidates to address questions regarding the evolutionary history of animal metaorganisms, in general, and bilaterians, in particular, besides research investigating the evolutionary origin of organismal processes and complexity.

In *Hydra*, bacteria colonize the mucus-like layer covering the ectoderm of the polyp and provide protection for the metaorganism against fungal infections (Fraune et al., 2015). The use of gain-of-function and loss-of-function approaches in *Hydra* has proven that these specific bacteria are selected by species-specific antimicrobial peptides that are secreted by both epithelial cells (Franzenburg et al., 2013) and neurons (Augustin et al., 2017). These facts have led to the hypothesis that both the innate immune system and the nervous system have evolved to orchestrate multiple functions including host–microbiome interactions (Bosch, 2013; Klimovich and Bosch, 2018). The isolation and development of genetically manipulated

variants in *Curvibacter* (Wein et al., 2018), the main colonizer of *Hydra*, now allows functional studies on both the host and the bacterial symbiont.

In parallel with model systems and model organisms, non-classical model systems may be developed to provide fundamental insights about ecologically important species that are otherwise hard to study or expensive to maintain in laboratory settings. For instance, stony corals and the reef ecosystems they build are in unprecedented decline due to local and global anthropogenic pressures, but they are also intrinsically complex systems and thus hard to study (as outlined in Voolstra, 2013). By comparison, the sea anemone *Aiptasia* is simple and inexpensive to rear, can establish symbioses with many of the same algal endosymbionts (Hambleton et al., 2014), and associates with some of the same bacteria as corals do (Röthig et al., 2016). Importantly, insights gained from such emerging model systems need to be confirmed in ecologically relevant target species in their native environment. As an example, salinity-conveyed thermotolerance and decreased bleaching (i.e., loss of algal endosymbionts) has recently been shown for a group of symbiotic *Aiptasia* anemones (Gegner et al., 2017). Elucidation of the underlying mechanism showed that the concentration of the oxygen-scavenging osmolyte floridoside, which is produced by the algal endosymbionts, is increased at high salinity, and reactive oxygen species (ROS) leakage, one of the hallmarks of coral bleaching, is reduced (Ochsenkühn et al., 2017). Thus, model systems can contribute to understanding climate change effects, even before working directly with ecologically relevant species.

5. Conclusion: reductionist and integrative approaches are needed to tackle the complexity of the metaorganism

The metaorganism framework challenges our understanding of self and non-self in some ways, particularly with regard to extended phenotypes and the nature of selectable units (Rees et al., 2018), and raises the question what level of reduced complexity or biological relevance may still be meaningful for experiments and assessments of functional roles. Depending on the research question at hand, a decision needs to be made whether the focus of the study should be on the target organism *sensu stricto* or whether consideration of the extended metaorganism is warranted.

We suggest that both reductionist and integrative approaches are necessary for understanding the scope of organism and metaorganism function (Fig. 2). While it is possible to understand many aspects of the biology of an organism without considering its associated microbes, we will not be able to comprehensively understand the biology of an organism in its ecosystem context without taking this factor into account. That is to say, the study of metaorganisms (in their ecosystem context) can provide broader insights into biological function than can be obtained by studying their individual components using a reductionist approach. As such, developing a suite of metaorganism model systems is necessary for targeting metaorganisms of ecological relevance, such as reef-building corals. Therefore, model systems such as *Aiptasia* (Baumgarten et al., 2015) for studying the dinoflagellate–cnidarian endosymbiosis or *Nematostella* (Fraune et al., 2016) for studying the function of bacteria are rapidly being developed in an effort to understand the mechanistic underpinnings of reef-building corals in order to mitigate the loss of global coral reef cover.

Even with the adoption of novel study species, model systems will continue to be an important tool. They are chosen because they allow researchers to study a specific biological phenomenon or because they are representative members of a particular lineage. However, when using model organisms or model systems one still has to integrate all gained insights across a broader range of species in their native environments and with their native ecological interactions. Every organism is unique, and insights from model organisms

– however useful those insights may be – can only be taken as rough guides as to how the organism of interest functions. Finally, metaorganisms should be chosen based on their ecological impact. For instance, the comb jelly *Mnemiopsis leidyi*, originally from the East coast of the Americas, is now found throughout Western Eurasia (Jaspers et al., 2018) and is currently being developed as a model system to study the biology and ecology of marine invasive species. Its vast expansion over the last decades led to strong ecosystem impacts in invaded areas, such as a decrease in zooplankton standing stock, a decline in pelagic fish recruitment, and oxygen depletion (Kideys, 2002). At present, the contribution of associated bacteria to its invasion success is not yet known. However, its broad tolerance to abiotic factors (e.g., salinity level, water temperatures), which characterizes many invasive species, might be in part attributable to microbiome adaptation. Thus, understanding the factors which contribute to the success of non-indigenous species from a metaorganism perspective holds great promise for understanding their differential success. Taken together, the metaorganism perspective is a powerful new framework which may be used to address long-standing biological questions such as the evolution and ecology of organismal complexity and the importance of organismal symbioses to ecosystem function. At the same time, and despite the integrative holistic view of organisms dictated by the metaorganism frontier, only reductionist approaches can untangle the complexity of the metaorganism. Such reductionist approaches are urgently required to clarify the nature of the interactions between microbes and their animal, plant, and fungal hosts.

Author contributions

Conceptualization by CJ, DM, TCGB, CRV. CRV and CJ wrote the manuscript, with contributions from SF, AEA, DJM, TCGB. Figures 1 and 2 were conceived by CRV, with input from CJ and SF. All authors reviewed and approved the final manuscript.

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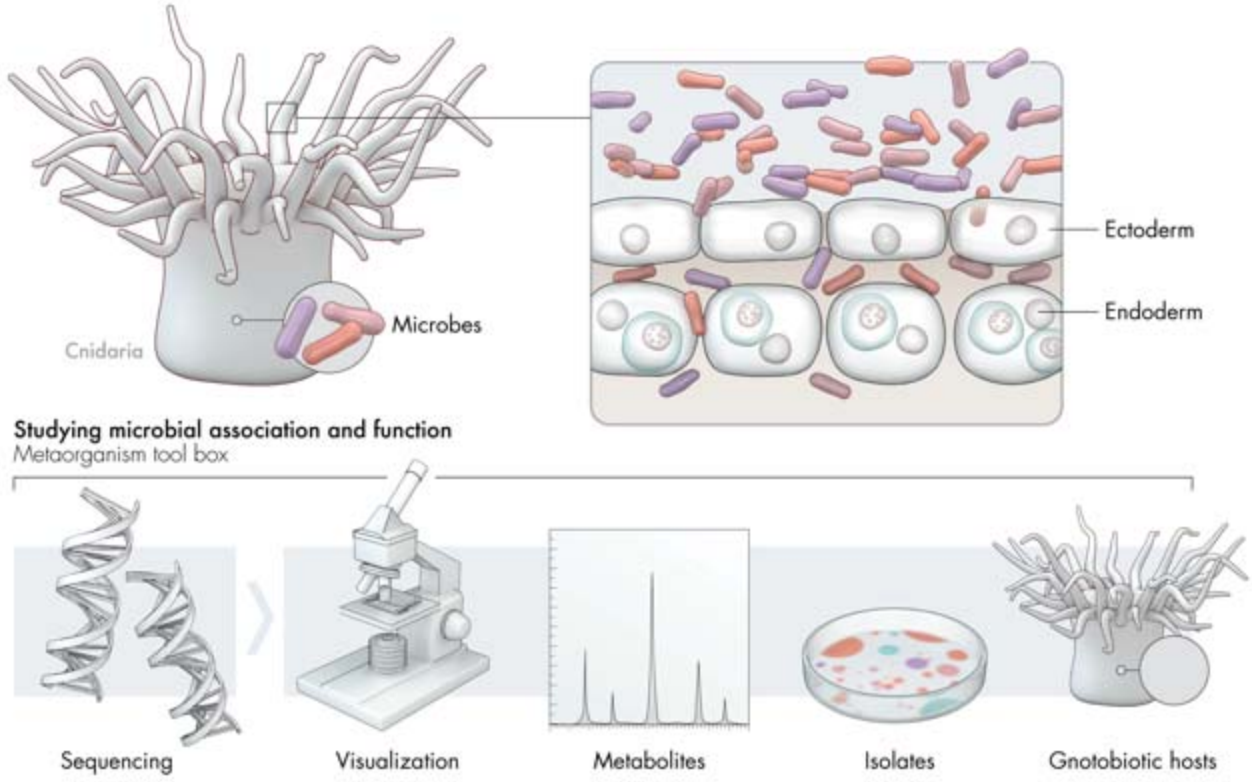

Fig. 1. Microbes in and around us and the toolbox available to study them. The metaorganism framework highlights the functional dependence between eukaryotic hosts and their associated microbes. A diverse set of methods (the metaorganism toolbox) is available to study microbial association and function in order to complement any initial description of microbe diversity via marker gene sequencing.

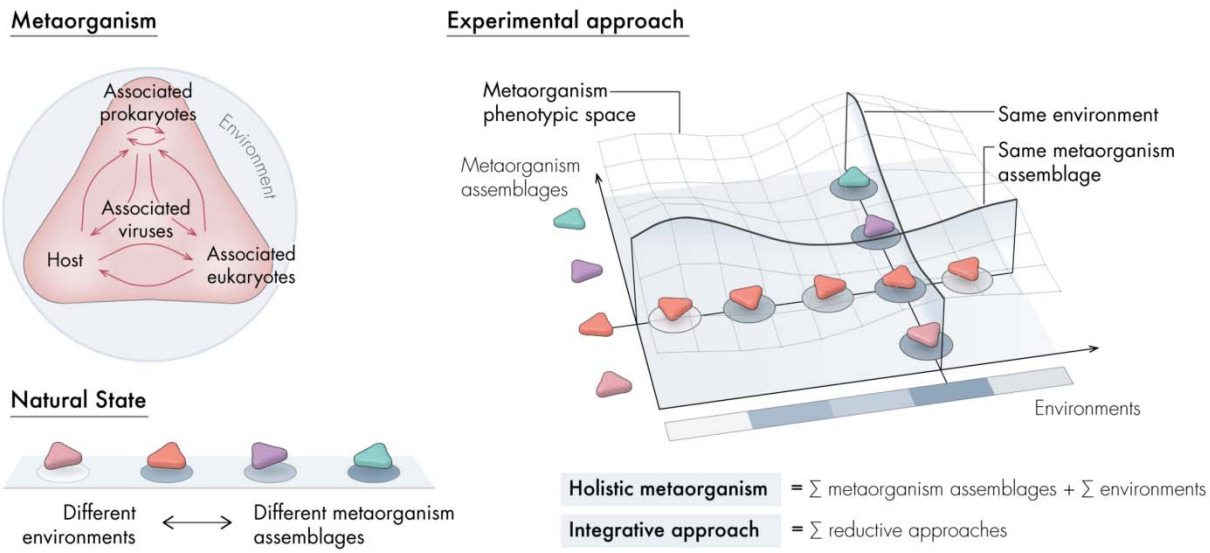

Fig. 2. The importance of reductive and integrative approaches for gaining a holistic understanding of the metaorganism. The metaorganism is composed of the host and its associated eukaryotes, prokaryotes, and viruses that comprise a unit surrounded by a common environment. Notably, host-microbe associations are not static and may differ with regard to host developmental stage, age, reproductive state, or physiological condition. As such, different metaorganism assemblages may be found in different environments. This 'fluidity' needs to be acknowledged in the experimental approach, where the complexity of the metaorganism is illustrated by its potential phenotypic space (square area), which is a function of the different environments (x-axis) and metaorganism assemblages (y-axis), i.e. microbes that the host associates with. Notably, different metaorganism assemblages in different environments display different fitness, which is denoted by peaks and valleys in the metaorganism phenotypic space. A reductionist approach can help divide this space into smaller 'slices' or 'units' by either considering the same metaorganism assemblage in different environments (moving along the x-axis) or by considering different metaorganism assemblages in the same environment (moving along the y-axis), and combinations thereof (moving diagonally). The intersection points of the x- and y-axes denote the host *sensu stricto*. The holistic metaorganism is elucidated by understanding the sum of all metaorganism assemblages in all habitable environments, and the integrative approach follows as the sum of all reductive approaches.