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## Metaorganisms as the new frontier

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### Summary

Because it appears that almost all organisms are part of an interdependent meta-organism, an understanding of the underlying host-microbe species associations, and of its evolution and molecular underpinnings, has become the new frontier in zoology. The availability of novel highthroughput sequencing methods together with the conceptual understanding that advances mostly originate at the intersection of traditional disciplinary boundaries, enable biologists to dissect the mechanisms that control the interdependent associations of species. In this perspective article, we outline some of the issues in interspecies interactions, present two case studies illuminating the necessity of interfacial research when addressing complex and fundamental zoological problems, and show that an interdisciplinary approach that seeks to understand co-evolved multi-species relationships will connect genomes, phenotypes, ecosystems and the evolutionary forces that have shaped them. We hope that this article inspires other collaborations of a similar nature on the diverse landscape commonly referred to as “zoology”.

### Interfacial research to thoroughly understand host-microbe species interactions

In physics, the interface between two phases has very different properties from that of the bulk phase and is important in a variety of processes; many natural and technological processes involve phenomena dominated by interfacial mechanics, that is, occurring within the regions of intersection between several fluid and /or solid phases. In contemporary zoology, specialization still has its place but the dynamics of interdisciplinary approaches appear central to approaching some of the toughest problems, such as a successful explanation of the biochemical basis of adaptations, the origin of morphological novelty, and a thorough understanding of species interactions, among and between members of the macro- and microbiota.

Technological advances driven by researchers representing multiple disciplines have altered our perspective on zoological questions. The potential uses and applications of next-generation sequencing technologies, for example, span the whole spectrum of ecological and evolutionary research and enable to approach the interplay between genes and environment.

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For many animal species genomic resources are available including genomic and cDNA libraries, microarrays, web-based bioinformatic portals, annotation and gene expression databases, and well-resolved phylogenetic frameworks. Extensive genomic and transcriptomic analyses of single cells (using whole genome amplification) to entire microbial communities (using metagenomics and metatranscriptomics) are now feasible because of ultrahigh throughput sequencing methods at extremely low costs. Obtaining the complete genomic sequence information of a whole ecosystem may be in reach within the next 10 years. In parallel to these advances in molecular techniques, novel high-resolution imaging methods, such as fluorescence techniques at subcellular resolution, digital-enhanced polarization, frequency and time resolved spectroscopic techniques, allow the quantitative analysis of cellular responses on external factors on the level of single proteins. A rapid expansion in the application of mathematics and analytical methodologies of computer science to biology allows advanced computational modelling and simulation to describe complex interacting systems in mathematical or statistical terms.

### Metaorganisms – a new term for an old concept

In 1877, Karl Möbius, Professor of Zoology at Kiel University (Figure 1), coined the term “biocenosis” for a community of living beings belonging to different species and associated by way of inter-species interdependence. In one of the first studies, later to become a classic, to be conducted in the emerging science of ecology, Möbius was seeking to determine why the oyster beds of Cancale, Marennes and Arca-chon were becoming exhausted, while the oyster beds in the British river estuaries and the Schleswig-Holstein oyster beds were very rich (Möbius, 1877). He related this phenomenon to the other species present, rather than to the oysters in the beds themselves. Möbius thus was the first to recognise that an ecological system must be taken as a whole and coined the term “biocenosis” for a living community. His biocenosis theory established itself as the basis of general ecology.

Today we realize that any multicellular organism must be considered a meta-organism comprised of the macroscopic host and synergistic interdependence with bacteria, archaea, fungi, and numerous other microbial and eukaryotic species including algal symbionts (Figure 2). We specifically refer to these associations as “metaorganisms”, because this collective term defines a superordinate entity that is applicable to all kinds of interdependent associations and the term is not constrained to specific taxonomic groups such as “holobiont” (usually used for cnidarians; for references see for example: Rohwer et al., 2002; Vega Thurber et al., 2009; Bourne et al., 2009) or “superorganism” (used for social insects such as ants; see for example Behmer 2009). Metaorganisms are polygenomic organisms. The term “metaorganism” was first used by Graham Bell (1998) to refer to organisms which are between two levels of organization. Recently, the term is increasingly used to refer to the totality of any multicellular organism derived from millennia of co-evolution with microbiota (Biagi et al., 2011). Even humans have been reviewed as ‘metaorganisms’ as a result of a close symbiotic relationship with the intestinal microbiota (Turnbaugh et al., 2007).

## Microbes as elements of a forgotten organ

From protists to humans, all animals and plants are inhabited by microbial organisms. Most of life's diversity in fact originates from microbial organisms. It is therefore remarkable that the microbial world is so little recognized by the zoologist except in the context of animal or plant disease and environmental decomposition. The reason for the general neglect is obvious: perception of the microbial world usually requires observation of the consequences of microbial activities, which can be subtle and difficult to investigate experimentally (McFall-Ngai, 2002; 2008). However, there is an increasing appreciation that microbes are an essential part of the animal phenotype influencing fitness and thus ecologically-important traits of their hosts (O'Hara AM and Shanahan F, 2006; McFall-Ngai, 2007; Fraune and Bosch, 2010). Macroscopic manifestations of microbial activities in animal development can be found in an increasing number of invertebrate and vertebrate models (Figure 3). Now, with an array of high-resolution technologies, first insights into the crosstalk between animal hosts and their microbial symbionts for maintaining a long-term coexistence have been reported (Wier et al., 2010).

Many animals carry symbiotic microbes that provide defense against natural enemies (Gill-Turnes et al., 1989; Arnold et al., 2003; Jaenike et al., 2010). In *Drosophila*, for example, the endosymbiont *Spiroplasma* rescues *D. neotestacea* females from the sterilizing effects of nematode parasitism. In vertebrates, Chytridiomycosis, a fungal disease caused by *Batrachochytrium dendrobatidis* (*Bd*), has caused many amphibian declines and extinctions. Resistance in amphibians unaffected by *Bd* is in part due to the presence of antifungal microbial species on the skin. Lisa Belden's lab at Virginia Tech in Blacksburg has recently isolated the bacterial species *Janthinobacterium lividum* from three temperate amphibian species including *Rana musoca* and shown in vitro that it inhibits *Bd* by secreting violacein, an antimicrobial metabolite, at low concentrations (Jenifer Walke, pers. commun.). Members of the normal human microbiota also inhibit pathogen colonization in human: *Staphylococcus epidermidis*, for example, inhibits nasal colonization by *Staphylococcus aureus*, a human opportunistic pathogen (Iwase et al., 2010) via a serine protease. From an evolutionary perspective, these examples demonstrate that in natural populations defensive symbionts must be considered as major players in the ecology of species interactions (Gill-Turnes et al 1989; Arnold et al., 2003; Oliver et al., 2003; Scarborough et al., 2005; Hedges et al., 2008; Teixeira et al., 2008; Iwase et al., 2010).

Bacteria also must be seen as essential part of the vertebrate immune system. The paradigm that the adaptive immune system has evolved to control microbes has been modified to include the concept that the immune system is in fact controlled by microorganisms (McFall-Ngai, 2007; Eberl, 2010). For instance, access for microbiota to the adaptive immune system in mammals has beneficial consequences for the host, in that antibacterial T<sub>H</sub>17 and T<sub>H</sub>1 responses are generated that are known to control pathogenic bacterial pathogens (Ivanov et al., 2008; Ivanov et al., 2009). The induction of TH17 and TH1 responses in the gut is closely associated with the induction of regulatory T cell subsets (Chaudhry et al., 2009). These responses are known to have beneficial effects for survival of long-lived chronic helminth infections in the absence of overt pathology and indeed are central to the tenet of the modified hygiene hypothesis (Yazdanbakhsh & Matricardi, 2004).

The diverse interactions reported between the mammalian gut and its microbiota include not only the control of the immune system but also cooperation in food breakdown (e.g. Hooper et al. 2002), participation in the regulation of fat accumulation (Bäckhed et al. 2004), and the induction of specific gene expression in the intestinal cells (e.g., Cash et al., 2006); microbes are also needed for the formation of the villi capillaries (Stappenbeck et al. 2002) and gut-associated lymphoid tissue (Bouskra et al., 2008).

## Species interactions moderate evolutionary processes

A strictly microbe-dependent life style has profound evolutionary consequences – and implies that the phenotype of a healthy animal cannot be explained entirely by its genome. Based on field and laboratory observations indicating that corals can adapt rapidly to changing environmental conditions by altering their population of symbiotic bacteria (Kushmaro et al., 1996; Koren and Rosenberg, 2006; Rosenberg et al., 2007), a group of scientists at Tel Aviv University proposed (Reshef et al. 2006) that a dynamic relationship exists between symbiotic microorganisms and environmental conditions that brings about the selection of the most advantageous coral holobiont. The hologenome theory of evolution (Zilber-Rosenberg and Rosenberg, 2008) considers the holobiont with its hologenome as a unit of selection in evolution. While the holobiont is defined as the host organism and all of its symbiotic microbiota, the hologenome is the sum of the genetic information of the host and its microbiota. The theory is based on the facts that all animals and plants harbour abundant and diverse microorganisms; that symbiotic microorganisms are transmitted between generations; and that under environmental stress, the symbiotic microbial community can change rapidly. The hologenome theory suggests that microbial symbionts affect the fitness of the holobiont and play an important role both in adaptation and in evolution of higher organisms. Genetic variation in the holobiont that can occur either in the host and/or in the microbial symbiont genomes can then be transmitted to offspring. In addition to the known modes of variation, i.e., sexual recombination, chromosomal rearrangement and mutation, variation in the holobiont can occur also via two mechanisms that are specific to the hologenome theory: amplification of existing microorganisms and acquisition of novel strains from the environment (Rosenberg et al., 2009). Thus, rapid changes in the symbiotic microbiota could allow the holobiont to adapt and survive under changing environmental conditions. But why do multicellular organisms have such different microbial communities? And how much do they matter? Do different organisms have distinct microbial signatures at birth, or do they evolve as the organisms age? While these questions remain to be answered, integrative and interdisciplinary approaches in two invertebrate metaorganisms, corals and aphids, have uncovered surprising links between host-microbe interactions and defense strategies.

### Case study 1: Species interactions in corals

Coral tissue and coral mucus contains abundant and highly complex microbial communities (Dinsdale et al., 2008 a; Dinsdale et al., 2008b; Kvennefors et al., 2010; Sharp et al., 2010). Warming waters are triggering coral bleaching and disease in the Caribbean, Indian Ocean and Great Barrier Reef off the Australian coast. Coral bleaching is accompanied by drastic shifts in the microbial community factors including changes in the production of

antimicrobial compounds (Kushmaro et al., 1996; Frias-Lopez et al., 2002; Rohwer et al., 2002; Ritchie, 2006; Bourne et al. 2008; Rosenberg et al., 2009). However, up to now there is little if any understanding about the the coral-microbe interactions and the underlying mechanism for what causes coral disease and bleaching (Ritchie, 2006; Ainsworth et al., 2008; Kvennefors et al., 2008).

It needed an interdisciplinary lab headed by an evolutionary ecologist and research combining mathematical simulations and collaboration with experimental biologists to identify the mechanisms that drive the dynamics of species interactions in the coral reef ecological system (Mao-Jones et al., 2010). The researchers from Cornell University used models to simulate bacterial community dynamics within the surface coral mucus under normal conditions and under warmer conditions. The model reveals for the first time how a healthy, normal microbial community in the coral surface-mucus layer protects corals from disease by preventing the invasion and overgrowth of pathogenic bacteria. When corals are stressed by warmer temperatures, there is a critical threshold where the community of microbes suddenly switches. Species associated with a healthy coral organism -- "resident species" -- decline as pathogens associated with coral disease take their place. The new model also added support to the previous observation that once the disease-causing microbes establish themselves, they persist even if the water cools down enough to favor the beneficial bacteria. The coral is then often too damaged to recover, and the reefs begin to die. In sum, by uniting researchers from multiple disciplines for the first time, there is an explanation how beneficial bacteria on coral suddenly give way to pathogens when waters warm.

## Case study 2: Species interactions in aphids

Pea aphids (Figure 3 E) and their symbiotic microorganism *Buchnera aphidicola* have been co-evolving for about 150 MY. Aphids feed on plant juices that they obtain from the phloem tissue of leaves and stems using long piercing mouthparts. Phloem is rich in carbohydrates, but low in the nitrogenous compounds that complex organisms need to make proteins to survive. Symbiotic *Buchnera* bacteria living inside the aphid provide these missing proteins. In addition to providing essential amino acids that are scarce in its diet, coupled purine metabolism of aphid and *Buchnera* contributes to the dependence of the pea aphid on this symbiosis (Ramsey et al., 2010). This symbiosis is uniquely amenable to global analyses of interactions between animals and their resident microbiota since it is the only animal symbiosis for which the genomes of both the host and its bacterial symbiotic partners have been sequenced (International Aphid Genomics Consortium (2010); <http://www.iagc.org>). "Metaorganismal genomics" now opens new roads to understanding the adaptive processes of these animals to adverse conditions.

During the millions of years that these two symbiotic partners have evolved, both the aphid genome and the *Buchnera* genome has undergone major genomic changes as a result of adapting to intracellular life (reviewed in Brinza et al., 2009). New research suggests (Nikoh et al. 2010) that the aphids' ability to host *Buchnera* depends on genes they acquired from yet another species of bacteria via lateral gene transfer (LGT). Most transferred genes were closely related to genes from relatives of *Wolbachia* (Alphaproteobacteria) indicating that

aphids utilize a set of duplicated genes acquired from other bacteria in the context of the *Buchnera*-aphid mutualism. Taken together, these findings impressively demonstrate the interweaving of organisms and their genomes over time and their merging in different ways.

One of the most unexpected findings of the aphid genome sequencing project was the absence of many genes involved in defending the insect from pathogens, parasites and predators. This was surprising as pea aphids are attacked by a variety of natural enemies ranging from fungal pathogens to parasitoid wasps. The aphids vary in their resistance to the wasps, which scientists previously had chalked up to genetic differences between aphids. Unexpectedly, however, Nancy Moran and colleagues in the University of Arizona's department of entomology (now at the Department of Ecology and Evolutionary Biology at Yale University) have shown that the wasp-resistant aphids owe their lives not to a specific genetic predisposition but to facultative symbiotic bacteria *Hamiltonella defensa* carried inside them (Oliver et al., 2003; Oliver et al., 2005; Oliver et al., 2009; for review see Oliver et al 2010). Such a newly acquired resistance is heritable, because the bacteria get passed down from mother to her offspring (Moran et al., 2005). Consistent with the holobiont theory of evolution (Zilber-Rosenberg et al., 2008) this implies that aphids acquire resistance to natural enemies by picking up bacterial symbionts, rather than having changes in the aphids' genes. A truly transdisciplinary analysis performed by experts from a Department of Entomology, a Department of Biology, two laboratories of Magnetic Resonance and Atomic and Molecular Physics, an Institute of Physics and Mathematics, a School of Biology and Environmental Science, a Department of Biomolecular Medicine, and a Department of Surgery and Cancer, deepened our understanding of the interaction between aphids and *B. aphidicola* by uncovering the central role of amino acid metabolism in the aphid – *Buchnera* symbiosis (Wang et al., 2010).

### **A call for transdisciplinary collaboration in zoology**

Organisms do not live in isolation, but have evolved, and continue to evolve, in the context of complex communities and specific environmental conditions. The two case studies presented here show that concerted multidisciplinary efforts are required in the future to examine the relationship between multiple phenotypes and the environmental context of organisms. Evolutionary biologists are increasingly able to integrate information across many organisms, from multiple levels of organization and about entire systems to gain a new integrated understanding that incorporates more and more of the complexity that characterizes interdependent species associations. We are at the beginning of what one may call a fundamental shift in zoological research. As zoology becomes more interdisciplinary in its practice and interdisciplinary groups are increasingly the order of the day, with advances being made at the intersection of traditional discipline boundaries, the metaorganismal structure of animals should provide a promising searching ground, for some principles of metaorganismal organization must transcend the particular organism that happens to occupy a given role at any moment. We predict that within the next 5 years genomics and ecology will get closely connected. And that only when we begin to understand the molecular base for adaptation and interactions of *Lebensgemeinschaften* (communities of life, Karl Möbius) can we start to comprehend how ecosystems are functioning. Currently we do not yet know the rules of composition of meta-organisms. We



do not even know if rules exist in the usual sense. We take the examples presented as auspicious beginnings that trace evolution's complex fingerprints by pioneering new kind of collaborative research.

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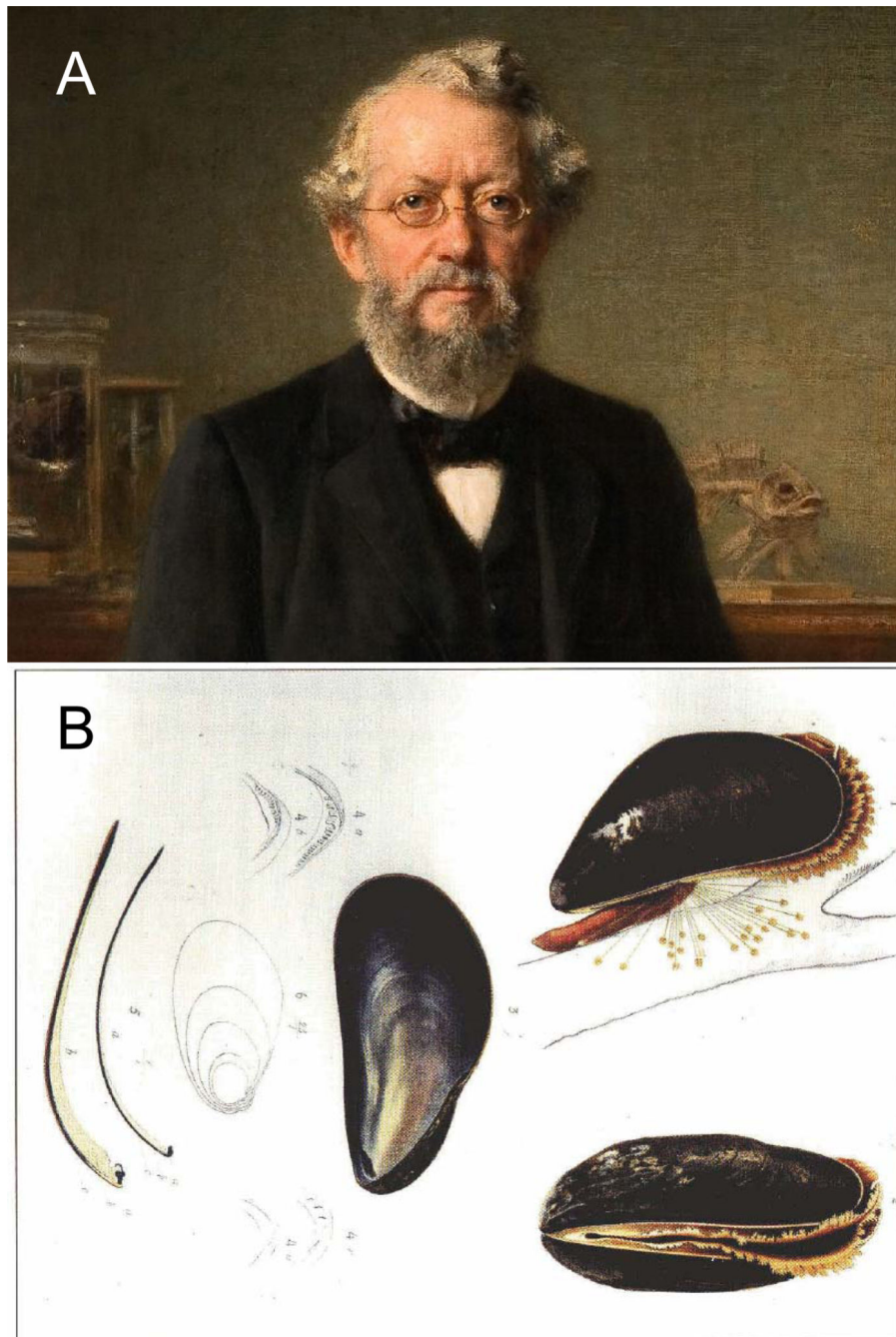
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**Figure 1. Karl August Möbius and the biocenosis concept**

(A) Karl August Möbius, Gemälde von Ernst Hildebrand, 1895. (B) Möbius' study object: the molluscs in the North Sea. From: H. A. Meyer, K. Möbius: *Fauna der Kieler Bucht*. Erster Band: Die Hinterkiemer oder Opisthobranchia. Zweiter Band: Die Prosobranchia und Lamellibranchia nebst einem Supplement zu den Ophistobranchia. Engelmann. Leipzig 1865–1872

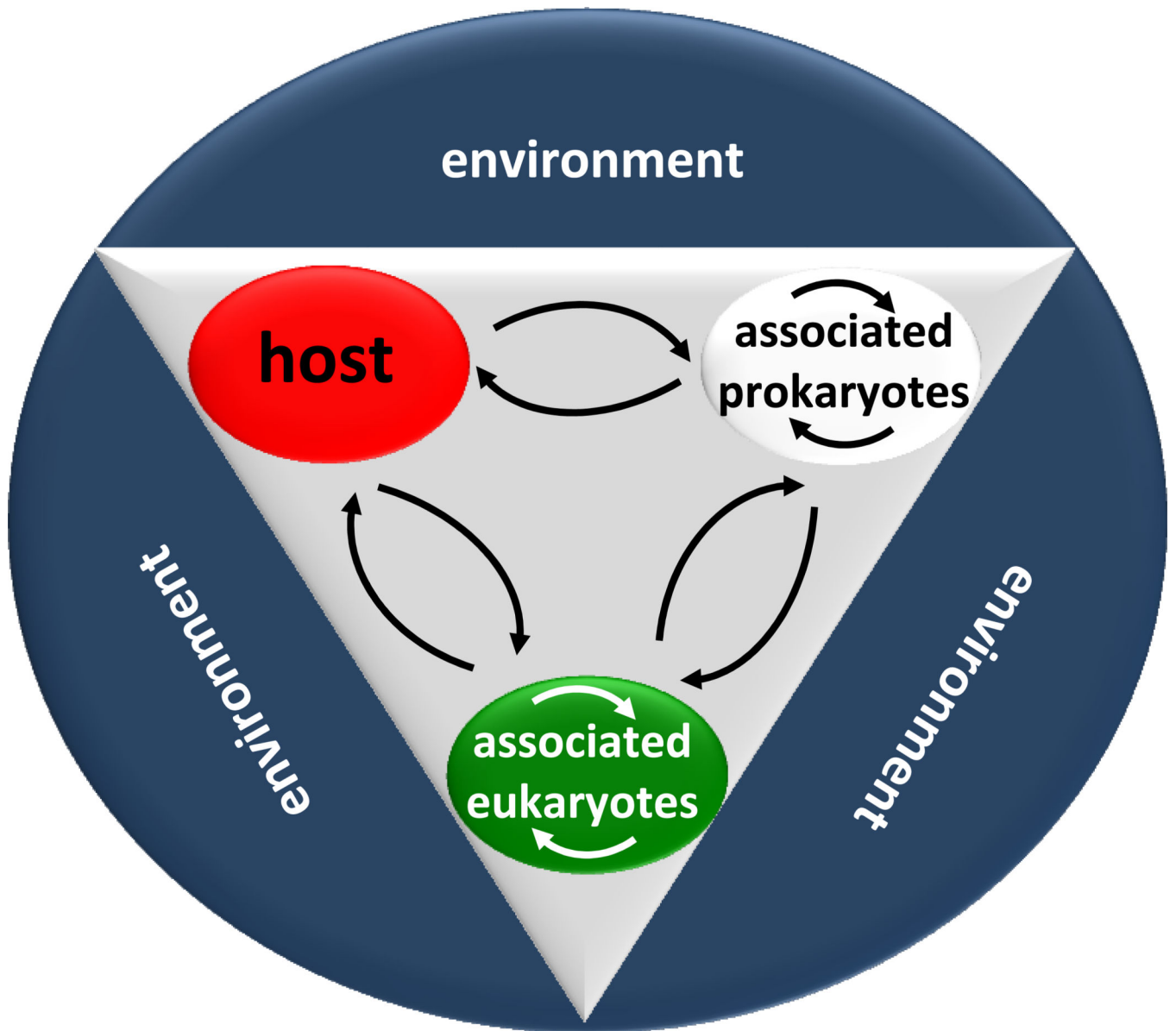
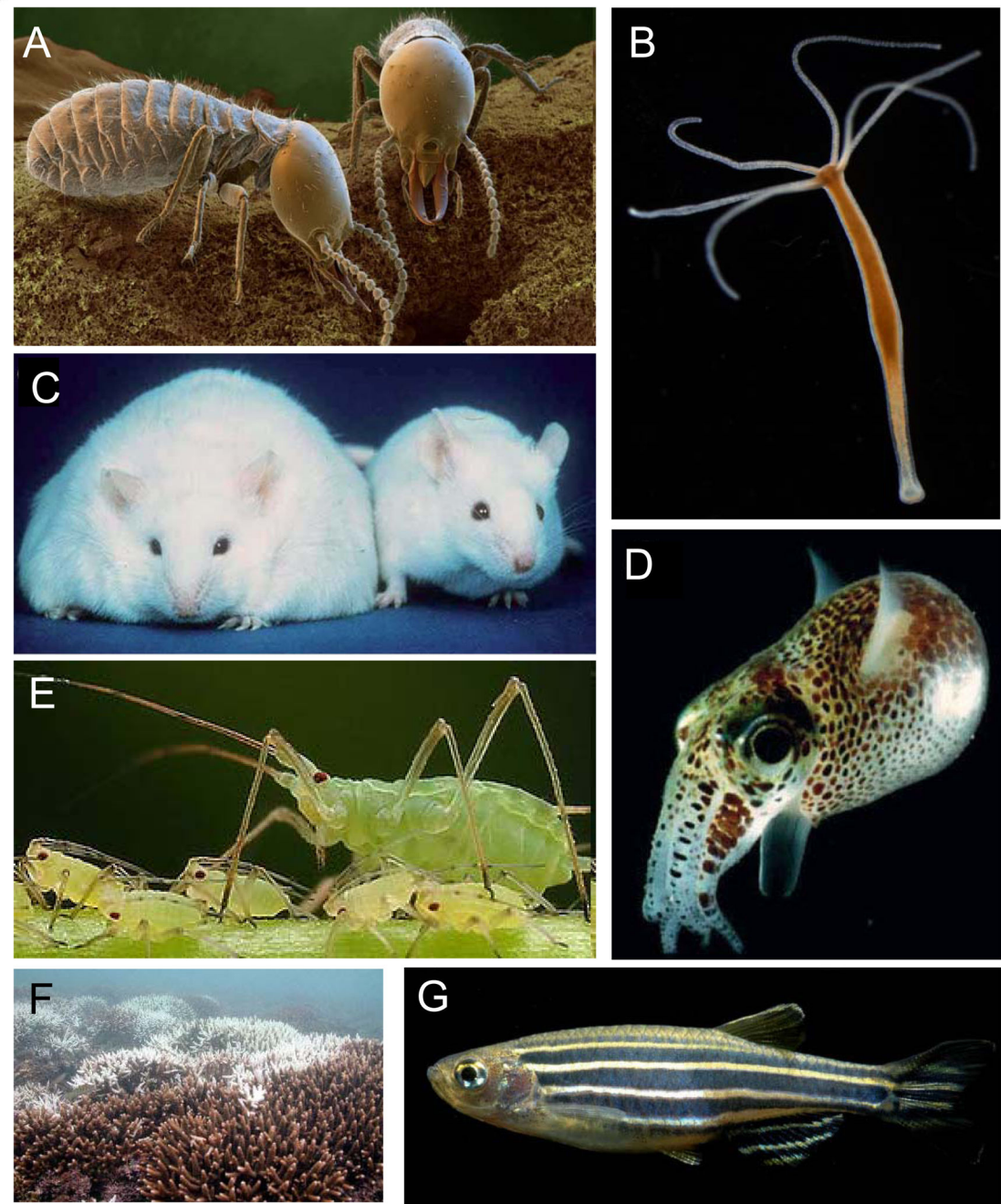


Figure 2.

**Multicellular organisms are metaorganism** comprised of the macroscopic host and synergistic interdependence with bacteria, archaea, fungi, and numerous other microbial and eukaryotic species including algal symbionts.





**Figure 3. Selected model organisms allow integrated analysis of host-symbiont interactions**

(A) Termites live in symbiosis with cellulose-degrading gut microbes (© Bayer). (B) Hydra is a new model organism for epithelial host-microbe interactions. (C) Pathogenic effects of a disturbed host-microbe homeostasis have been discovered in mice (© ONRL). (D) Aphids harbor the symbiont *Buchnera aphidicola*, which supplies the host with essential amino acids, that are not included in the phloem sap diet (© Alex Wild). (E) The bacterium *Vibrio fischeri* induces the formation of the light organ in the squid *E. scolopes* (picture taken from M. J. McFall-Ngai and E. G. Ruby). (F) The coral bleaching disease is a

global threat of coral reefs. Changes in the microbiota of the coral host can confer resistance to bleaching (© Lee James Pantas).

(G) The gut development of the zebrafish *Danio rerio* is dependent on the presence of gut bacteria.