

# 1 **Host-associated microbiomes and their roles in marine ecosystem functions**

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35

## 36 **Abstract**

37

38 The significance of mutualisms between eukaryotic hosts and microbes extends from the  
39 organismal to the ecosystem level, and mutualistic symbioses underpin the health of Earth's most  
40 threatened marine ecosystems. Despite rapid growth in research on host-associated microbes  
41 (microbiomes), very little is known about their interactions for the vast majority of marine host  
42 species. We outline research priorities to broaden our current knowledge of host-microbiome  
43 interactions and how they shape marine ecosystems. We argue that this research frontier will  
44 allow us to predict responses of species, communities, and ecosystems to stressors driven by  
45 human activity, and inform future management and mitigation.

46

## 47 **Introduction**

48

49 The marine environment is home to a dazzling diversity of macroscopic organisms including  
50 plants, animals, brown algae (*e.g.*, giant kelp), and others. Most of these taxa do not function  
51 entirely on their own but are covered, inside and out, with communities of microorganisms, also  
52 known as microbiomes [1]. There can be many types of functional interactions between hosts  
53 and their microbiomes. Here, we discuss mutualistic symbioses (long-term, persistent  
54 interactions between organisms where all partners benefit; see Box 1 for key terminology and  
55 concepts important for the discussion). Studies of marine organisms have provided many  
56 important examples of mutualistic symbioses, such as those in the bobtail squid [2], scleractinian  
57 corals, [3], sponges [4], shipworms [5], and chemosymbiotic invertebrates [6]. The work on most  
58 of these systems has been focused on a few specific host-associated microbial taxa in which a  
59 mutualistic role could be studied and tested in detail. However, recently there has been an  
60 explosion of studies of complex microbiomes with many different microbial species, rather than  
61 just one or a few microbes at a time [7]. Such studies have concluded that microbiomes strongly  
62 influence the function of their hosts [8]. In turn, the hosts play important roles in the structure

63 and function of diverse marine habitats [8,9] and mediate marine ecosystem-level responses to  
64 environmental change [10,11].

65  
66 Here, we report on the challenges and opportunities in studies of marine eukaryote-microbiome  
67 mutualisms. First, we highlight the limited extent of known ecosystem functions of host-  
68 associated marine microbes. Second, we outline ways in which comparative and experimental  
69 studies across hosts and habitats could be integrated to show how microbial symbioses contribute  
70 to host evolution, resilience, and conservation strategies. We list outstanding questions in  
71 ecology and evolution that could be addressed by expanding the phylogenetic and ecological  
72 breath of host-associated microbiome studies, including all possible mutualistic interactions  
73 throughout the microbiome, and we give specific examples of how these questions could be  
74 answered. There is strong empirical evidence and new consensus that biodiversity (*i.e.*, the  
75 diversity of organisms and their interactions) pervasively influences the functioning of Earth's  
76 ecosystems, including increasing productivity [12,13]. However, this research has focused  
77 almost exclusively on macro-organisms. Because microbial symbionts are integral parts of most  
78 living organisms (*i.e.*, an extended phenotype [14]), broadening our understanding of how  
79 microbial symbionts contribute to host performance and adaptability is essential.

80

### 81 **How microbial symbiosis impacts marine ecosystem functioning**

82

83 *Foundations of productive ecosystems.* Ecosystem engineers such as corals, deep-sea mussels,  
84 and hydrothermal vent tubeworms contribute to primary productivity and create the structural  
85 habitats and nutrient resources that are the foundation of their respective ecosystems [15]. All of  
86 these taxa engage in mutualistic nutritional symbioses with microbes. There are many examples  
87 of marine nutritional mutualisms where microbes enable hosts to utilize resources or substrates  
88 otherwise unavailable to the host alone. Such symbioses have been described in detail in reduced  
89 and anoxic sediments (*e.g.*, lucinid clams, Stilbonematid nematodes, and gutless oligochaetes)  
90 and hydrothermal vents (*e.g.*, the giant tube worm *Riftia pachyptila* or *Bathymodiolus* deep-sea  
91 mussels) [8]. Many foundational species of marine macroalgae are vitamin auxotrophs (for  
92 example, half of more than 300 surveyed species were unable to synthesize cobalamin), and their  
93 productivity depends on provisioning from their epiphytic bacteria [16]. On coral reefs,

94 *Epulopiscium* bacteria in the guts of surgeonfishes produce enzymes that allow their hosts to  
95 digest complex polysaccharides, enabling the host fish to feed on tough, leathery red and brown  
96 macroalgae [17]. This trophic innovation has facilitated niche diversification among coral reef  
97 herbivores. Surgeonfishes are critical to the functioning of Indo-Pacific coral reefs, as they are  
98 among the only fishes capable of consuming large macroalgae that bloom in the wake of  
99 ecosystem disturbance and suppress coral recovery [18].

100

101 *Reproduction, development, and interaction networks.* Extending beyond nutritional symbioses,  
102 symbionts can alter the reproduction, development, and growth of their hosts. Specific bacterial  
103 strains in marine biofilms often directly control the recruitment of planktonic larvae and  
104 propagules, either by inhibiting settlement or by serving as a settlement cue [19,20]. For  
105 example, the settlement of zoospores from the green alga *Ulva intestinalis* onto the biofilms of  
106 specific bacteria is mediated by their attraction to the quorum sensing molecule, acyl-homoserine  
107 lactone, secreted by the bacteria [21]. Classic examples of marine host-microbe developmental  
108 dependence include the observation that algal cultures grown in isolation exhibited abnormal  
109 morphologies [22] and the subsequent discovery of morphogenesis-inducing compounds, such as  
110 thallusin, secreted by epiphytic bacterial symbionts [23]. Bacteria are also known to influence  
111 the growth of marine plants, macroalgae, and phytoplankton by secreting phytohormones such as  
112 indole acetic acid and cytokinin-type hormones [24–26]. In the marine choanoflagellate  
113 *Salpingoeca rosetta* both multicellularity and reproduction are triggered by specific bacterial  
114 cues, offering a view into the origins of bacterial control over animal development (reviewed by  
115 Woznica and King [27]). Perhaps the best studied example of intimate host-microbe interactions  
116 controlling animal development is the Hawaiian bobtail squid *Euprymna scolopes* [28]. It lives  
117 in a mutualistic symbiosis with the bioluminescent bacteria *Aliivibrio fischeri*. The bacteria are  
118 fed a sugar and amino acid solution by the host and in return provide bioluminescence for  
119 camouflage and possibly also for attracting prey [2]. This mutualism with microbes provides a  
120 selective advantage for the squid in predator-prey interactions. Bioactive compounds produced  
121 by symbiotic bacteria often play a role in chemical defense for the host, as demonstrated in  
122 several species of bryozoans [29], sponges [30], molluscs [31], and ascidians [32]. In some  
123 cases, symbiotically produced defense compounds are acquired and sequestered by specialized

124 predators, as recently described in the sea slug *Elysia rufescens*, which acquires microbial  
125 symbiotically produced kahalalide toxins from its algal prey *Bryopsis* [33].

126

127 *Biofouling and microbial community assembly.* Some host-associated microbes produce  
128 compounds that prevent biofouling and regulate microbiome assembly and maintenance in many  
129 marine organisms, including sponges, macroalgae, and corals [34]. For example, tropical corals  
130 harbor diverse bacteria in their surface mucus layer that produce quorum-sensing inhibitors and  
131 other antibacterial compounds as a defense against colonization and infection by potential  
132 microbial pathogens. Epiphytic bacteria of marine macroalgae excrete a diverse chemical arsenal  
133 capable of selectively shaping further bacterial colonization and deterring the settlement of  
134 biofouling marine invertebrates such as bryozoans [24,35]. As in corals, these diverse,  
135 microbially-secreted compounds include not only bactericidal and bacteriostatic antibiotics, but  
136 also compounds like halogenated furanones, cyclic dipeptides, and acyl-homoserine lactone  
137 mimics that disrupt bacterial quorum sensing and inhibit biofilm formation [36]. While these  
138 prior examples illustrate how the microbiomes can protect hosts from surface colonization, a  
139 similar phenomenon has also been observed internally in the shipworm *Bankia setacea*, where  
140 symbionts produce a boronated tartrolon antibiotic thought to keep the wood-digesting cecum  
141 clear of bacterial foulants [37].

142

143 *Biogeochemical cycling.* Host-associated microbiomes also influence biogeochemical cycling  
144 within ecosystems with cascading effects on biodiversity and ecosystem processes. For example,  
145 microbial symbionts comprise up to 40% of the biomass of their sponge hosts. Through a process  
146 termed the “sponge-loop”, they convert dissolved organic carbon released by reef organisms into  
147 particulate organic carbon that can be consumed by heterotrophic organisms, helping explain  
148 Darwin’s paradox; *i.e.*, how highly productive coral reef ecosystems exist within otherwise  
149 oligotrophic tropical seas [38]. Some sponge symbionts likely also play a significant role in the  
150 marine phosphorus cycle by sequestering nutrients in the form of polyphosphate granules in the  
151 tissue of their host [39]. The sulfur-oxidizing gill endosymbionts of lucinid clams contribute to  
152 primary productivity through chemosynthesis and facilitate the growth of seagrasses, important  
153 foundation species, by lowering sulfide concentrations in tropical sediments [40].

154 *Gammaproteobacterial* symbionts of lucinid clams and Stilbonematid nematodes were also

155 recently shown to be capable of nitrogen fixation (bacterial symbiont genomes encode and  
156 express nitrogenase genes [41]), highlighting the role of symbiotic microbes in nutrient cycling  
157 in shallow marine systems. Many macroalgal-associated bacteria are specifically adapted to  
158 degrade complex algal polysaccharides (*e.g.*, fucoidan, porphyran, and laminarin [42,43]) and  
159 modify both the quality and quantity of organic carbon supplied to the ecosystem [44,45].  
160 Previously unrecognized metabolic innovations of marine microbial symbioses that are  
161 ecologically important are discovered regularly [46]. *Kentron* (a clade of *Gammaproteobacteria*  
162 found in association with ciliates) nourish their ciliate hosts in the genus *Kentrophoros* and  
163 recycle waste products from their hosts into biomass [47]. This symbiosis provides a  
164 counterexample to textbook descriptions of chemosymbiotic bacteria that make most of their  
165 biomass from fixing either CO<sub>2</sub> or methane.

166

167 *Environmental tolerance and resilience.* Individual taxa within the microbiome can also help  
168 hosts withstand a wide range of environmental conditions, including those predicted under  
169 scenarios of climate change. For example, in the marine nematode *Litoditis marina* species  
170 complex, members of the bacterial microbiome are hypothesized to confer broad tolerance to  
171 temperature and salinity ranges among the cryptic species (*i.e.*, distinct species that are  
172 morphologically very similar to each other) [48]. In oxygen-depleted deep-sea environments,  
173 bacterial symbionts associated with foraminifera appear to confer tolerance to hypoxia through  
174 varying metabolic pathways such as oxidizing hydrogen sulfide to detoxify the surrounding  
175 water [49]. In cultures of the filamentous brown alga *Ectocarpus*, the microbiome has been  
176 found to play an essential role in their adaptation to changes in salinity [50].

177

178 These examples demonstrate the importance of host-associated microbiomes for the functioning  
179 of present and future ocean ecosystems. Yet, such studies are few and efforts to link diversity  
180 and dynamics of complex marine microbiomes to organismal and ecosystem function promise  
181 important new insights. The role of symbiosis in ecosystem-level responses to change remains  
182 ripe for exploration, and indeed requires tools and approaches that consider entire microbiomes  
183 at once rather than one or a few symbiotic microbes.

184

185 **Example outstanding questions: The influence of microbiomes in a changing ocean**

186

187 Humans are altering the ocean environment and ecosystems by way of climate change,  
188 overexploitation, and pollution [51,52]. Knowledge about host-associated microbiomes may  
189 prove important in informing conservation in the face of change. Here we discuss how we can  
190 quantify the adaptive potential of host-microbe populations, and how this adaptation may affect  
191 ecosystem functions.

192

193 *How can microbial symbioses influence host adaptation in a changing ocean?*

194

195 Global change creates new environments and new conditions. Whether and how marine species  
196 adapt to change may depend on their microbiomes. Host-associated microbes can be treated as  
197 extended host phenotypes if host and microbe show a concerted adaptive response [53]. Vertical  
198 transmission of symbiotic microbes to the offspring is expected to stabilize the association  
199 between a given host and microbe, thereby making phenotypic traits of the host-microbe  
200 interaction potentially heritable. This stabilization could in turn drive adaptive evolution of host-  
201 microbe interactions if it allows host populations to adapt to new ecological niches or persist in a  
202 changing environment [14,54,55]. In contrast, horizontal transmission of symbiotic microbes  
203 generally requires some form of selective host filtering of beneficial symbionts and/or host  
204 sanctioning of detrimental symbionts and cheaters in order to align host and microbial interests  
205 and coordinate their adaptive responses [56]. Nevertheless, horizontally acquired bacterial  
206 symbionts have more opportunity to exchange genes with environmental bacteria and thereby  
207 increase their adaptive potential [57]. The acquisition of novel symbionts has been postulated as  
208 a novel form of phenotypic plasticity that could potentially assist foundational seagrass and  
209 macroalgal species acclimatize to a changing climate [58].

210

211 In most host-symbiont relationships where both kinds of transmission are possible, increasing  
212 levels of horizontal transmission will tend to decouple host and microbial interests, and vertical  
213 transmission will tend to bind them [59,60]. Understanding the role of microbial symbionts in the  
214 adaptive capacity of host populations requires transgenerational, functional, and comparative  
215 studies [54]. One source of novel insight into the mechanisms of adaptive evolution in microbial  
216 symbioses comes from comparative analyses of populations that recently radiated or diverged

217 through allopatric speciation events. Phylogenomic, transcriptomic, and metabolomic analyses of  
218 microbial symbionts associated with closely related sister species that are genetically isolated  
219 into contrasting environments holds the potential to uncover genes that coevolved with the host  
220 and other genes that diverged due to the changing environmental conditions [61]. The latter may  
221 shed light on the adaptability of host-microbe systems in a changing environment [62].

222

223 Several geological events offer opportunities to reveal drivers of adaptive evolution of hosts and  
224 their microbiomes. One model event is the formation of the Isthmus of Panama, which presents  
225 an unrivalled opportunity to study processes of diversification and adaptation in marine animals.  
226 Approximately 3 million years ago (Ma), after a previous ~20 million years (Myr) of tectonic  
227 and volcanic activity, the Isthmus of Panama formed a land bridge between North and South  
228 America, which prevented water from flowing between the tropical Atlantic and Pacific oceans  
229 [63]. Ocean currents became constricted and previously contiguous host populations and their  
230 associated microbiomes became isolated into the Tropical Eastern Pacific and Caribbean Sea  
231 (Figure 1, [63,64]). As the Caribbean and Pacific split, environments in the Caribbean changed  
232 from nutrient-rich and seasonally-variable [65] to stable and oligotrophic, which ultimately led to  
233 Caribbean-wide extinction events [65]. Caribbean survivors were well-adapted to low nutrient  
234 conditions and often radiated in response to the relatively-recent proliferation of Caribbean coral  
235 reefs [63]. Populations on both sides of the isthmus became physically isolated and followed  
236 separate eco-evolutionary dynamics in distinct environments [64]. The relatively recent  
237 emergence of islands (*e.g.*, Marquesas, Hawai'i or the Galapagos islands among others) presents  
238 similar natural events that can be used to study the coevolution of marine hosts and their  
239 associated microbes [66]. Animal communities on these islands, both terrestrial and coastal  
240 marine, include species that range from old to recent endemics (with or without sister species  
241 across barriers to dispersal), divergent populations, and populations that are still able to exchange  
242 genes across biogeographic barriers [67].

243

244 A great advantage of the Panamanian model system is the wealth of well-calibrated phylogenetic  
245 data available for a range of marine taxa (Fig. 1, [64,67]). For example, the numerous species  
246 pairs of snapping shrimps (genus *Alpheus*) that emerged through trans-isthmian vicariance (more  
247 than 10 pairs identified so far) have been used as a model system for understanding the genetic



248 and behavioral consequences of allopatric isolation. Extensive taxonomic and molecular work  
249 has identified a correlation between the timing of divergence and habitat use, whereby pairs  
250 occurring in shallower mangroves and intertidal habitats diverged later than pairs occurring in  
251 deeper coral reef habitats [68,69]. We hypothesize that parallel changes occurred to the  
252 microbiome at the taxonomic, functional or genomic level, driven by habitat and environment.  
253 Extending comparative analyses of microbiomes to species of urchins, porcelain crabs, snails,  
254 clams, and fishes, among others, across the Isthmus of Panama offers promise in revealing  
255 general processes of adaptive evolution (*e.g.*, loss/gains of genes in microbial genomes) and  
256 unveiling the relative contribution of vertical and horizontal transmission in marine host  
257 communities [70]. Moreover, overlaying data on microbial taxonomic and genomic composition  
258 on host phylogenies will help identify which microbes are tightly associated with the host, their  
259 mode of transmission, and their genomic features [71,72]. Ultimately, these comparative  
260 analyses should help identify and ultimately predict the short- and long-term responses of marine  
261 host-microbiome assemblages to ongoing environmental changes.

262

### 263 *How can we use marine host-microbe symbiosis studies to inform conservation?*

264

265 Human activities have been directly affecting the composition of natural microbiomes. Examples  
266 include the introduction of pathogens as well as non-native species and their microbial symbionts  
267 [73] and environmental contamination with antimicrobials in offshore farms [74]. Conventional  
268 aquaculture practices often promote high numbers of diverse bacteria, some of them symbionts  
269 and some pathogens, which in combination with the use of antibiotics can develop into hotspots  
270 for horizontal gene transfer [75] and consequent dissemination of antibiotic resistance [74]. The  
271 composition of host-associated microbiomes can also be modified by other stressors, such as  
272 elevated seawater temperatures associated with global climate change or the locally discharged  
273 water from power plants [76], oil spills [77], and contamination with heavy metals from mining  
274 activities [78], with potential effects on host biology. Mitigation strategies that make use of host-  
275 associated microbiomes by direct bioaugmentation (*i.e.*, enriching the environment with specific  
276 microbes) or through the biostimulation of specific metabolisms to enhance host resistance and  
277 recovery, have been promising, but are still rare. For example, corals exposed to high  
278 temperatures were significantly more resistant to bleaching when inoculated with a consortium

279 of microorganisms isolated from native healthy host corals [79]. Jin Song *et al.* (2019)  
280 summarized and discussed examples of successful probiotics used to promote animal health and  
281 conservation in the wild [80].  
282  
283 Effective microbially-based mitigation will benefit from a thorough understanding of the identity  
284 and physiology of beneficial microbes and the attributes of healthy microbiomes. To this end, the  
285 most successful trials of microbiome engineering considered niche specific traits and the  
286 manipulation of stable and native groups, rather than the use of generic microbial cocktails [81].  
287 Nevertheless, manipulative approaches can succeed even without knowing the detailed  
288 mechanisms *a priori*, as long as a rigorous experimental design is applied, which can eventually  
289 lead to the discovery of key strains and mechanisms [82]. From the perspective of applied  
290 ecosystem recovery, the most promising focal organisms are keystone and foundational  
291 organisms and their associated microbiomes. Efforts to quantify and compare the net effects of  
292 microbiome functions across multiple hosts and contexts (*e.g.*, health status, life-stage, and  
293 habitat) are critical to advancing our understanding of the roles of microbiomes for hosts and  
294 ecosystems [83]. As an example, microbiomes specific to different developmental stages in  
295 tropical corals suggest that different microbiomes serve distinct, specific roles throughout host  
296 life cycles [84]. One frontier for future studies of microbiomes is understanding the degree to  
297 which important functional roles can be maintained in non-optimal environmental conditions,  
298 and whether diverse communities of transient microbes may allow hosts to broaden their realized  
299 ecological niche [85]. Describing and understanding the organizing principles of microbiome  
300 assembly and maintenance is critical for effective microbial-based mitigation strategies.  
301 Studying shifts in microbiome taxonomic composition and functional diversity in organisms that  
302 experience drastic seasonal or thermal shifts (*e.g.*, temperate organisms or species living in  
303 intertidal zones) will help identify these principles [7,86]. For example, the microbiome of the  
304 temperate coral *Astrangia poculata* resembles a diseased tropical coral microbiome in the winter  
305 months, during host quiescence, and it transitions in the spring to a community dominated by  
306 taxa that continue to be present throughout the year [87]. This seasonal shift represents an  
307 opportunity to identify the molecular basis of microbiome assembly within an animal host. Such  
308 complex interactions among microbial species and their hosts can be informed by theory and  
309 empirical generalizations in community ecology developed primarily from studies of

310 macroorganisms, including succession, community assembly, metacommunities, multi-trophic  
311 interactions, disturbance, and restoration [7].

312

313 The role of microbiomes and the influence of host life stage and other external parameters on  
314 microbial functions is still poorly understood in most marine systems. To use microbiomes for  
315 informing management and conservation, we need background data on the natural dynamics of  
316 host-associated microbiomes across ecosystems. Well-replicated studies across spatial and  
317 temporal scales (*i.e.*, encompassing natural variation) and mesocosm experiments can help define  
318 the core microbiome and its flexibility in the face of environmental variations [88]. For example,  
319 a long-term study of corals showed that environmental perturbations can lead to transitions from  
320 stable to unstable community states, where diseased hosts show higher (and random) variation in  
321 microbial community composition compared to healthy individuals [89]. These results indicate  
322 that increased alpha-diversity in host-associated microbial communities in individual hosts does  
323 not always translate into healthier systems, and that a highly variable microbial assemblage is  
324 correlated to host vulnerability. Moreover, environmental stressors can compromise or eliminate  
325 beneficial microbe species that need to be replaced by beneficial, or at least neutral, microbes to  
326 passively prevent the spread of diseases.

327

### 328 **Integration of information across hosts for an ecosystem-level understanding of the roles of** 329 **microbial symbionts**

330

331 Future progress in research on host-symbiont interactions --and indeed on ecosystem functioning  
332 generally-- depends on adopting an entire microbiome perspective and expanding the scope of  
333 inquiry beyond single host taxa and individuals. First, this will require a broad comparative  
334 approach to identify similarities and differences across marine host species within a phylogenetic  
335 framework, especially with respect to their physiologies, microbiome profiles, and habitat  
336 distributions. Second, studies of terrestrial hosts and microbiomes can inform research priorities  
337 and generate hypotheses to be tested in marine environments [55,90]. Thus, we see great value in  
338 building a framework of broad collaborative networks. To promote such broad and collaborative  
339 efforts and to allow for transparent and reproducible research, we encourage researchers to share  
340 protocols, data, metadata, workflows (particularly data manipulation; *i.e.*, transformation and

341 quality filtering), and software. Collaborative efforts are more sustainable, and ultimately more  
342 productive, if we credit online resource generators, share data and workflows, and acknowledge  
343 others [91].

344  
345 Identifying the factors that promote the contribution of microbial symbionts to host adaptability  
346 is fundamentally important to understanding ecological and evolutionary processes as well as  
347 predicting the response of populations, species and communities in a changing environment. Key  
348 localities (*e.g.*, the Isthmus of Panama) can provide model systems to test hypotheses about the  
349 roles of marine host-associated microbiomes for ecosystem functioning. The biggest payback  
350 will likely come from a focus on taxa that have disproportionately large roles in the ecosystem,  
351 including dominant, foundation, and keystone species. We recommend special focus on how  
352 horizontally transferred microbes play critical roles in the hosts' ability to respond to  
353 environmental change. Together these research directions will enhance our ability to predict how  
354 climate change, invasion by non-native species, food-web disruption, and environmental  
355 contamination will play out, and inform practical strategies for directly assisting marine  
356 conservation in novel ways.

357  
358 **BOX 1. Terminology used to discuss communities of microbes and their interactions with**  
359 **hosts.**

360  
361 Following are key terms and concepts that we use in this paper (see also [92]).

362  
363 **Microbiome and microbiota.**

364 We use *microbiome* to refer to a community of microbes (organisms too small to see without the  
365 aid of a microscope) found at a specific place and/or a specific time. We avoid using the term  
366 *microbiota*, which has a complicated history (see [92]). Ideally, the place and/or time should be  
367 specified when discussing the microbiome. For example, “the seagrass microbiome” is the total  
368 of microbial communities found in association with seagrass and “the seagrass root microbiome”  
369 would be those microbial communities found in/on the roots of seagrass. We also consider *host-*  
370 *associated microbiomes* broadly to include any and all kinds of microbes (*e.g.*, bacteria, archaea,  
371 microbial eukaryotes or viruses), which can be transient or persistent, and have variable

372 functional impacts from beneficial to unimpactful to detrimental. Microbiomes can inhabit the  
373 external and internal surfaces of virtually every eukaryote, from microscopic unicellular diatoms  
374 to macroscopic organisms such as kelp, coral, seagrass, cephalopods, and vertebrates [88].

375

### 376 **Symbiosis.**

377 We use *symbiosis* here in the broad sense meaning a long-term, persistent relationship between  
378 two or more organisms in which at least one of them benefits. Symbioses come in three  
379 subcategories: in *mutualism*, both partners benefit; in *parasitism*, one partner benefits and the  
380 other is harmed; in *commensalism*, one partner benefits and the other is unaffected. In many  
381 discussions of microbes or microbial communities living in and on a host organism, it is  
382 frequently assumed that the microbe is benefitting in some way and the question then becomes,  
383 “What is the effect on the host?” If the host benefits, this is a mutualism, if the host is unaffected  
384 this is a commensalism, and if the host is harmed this is a parasitism. It is important to note that  
385 these categories are fluid in that the type of interaction between two species is often conditional  
386 and depends on many factors including genotype (of all partners), environmental conditions, and  
387 developmental stage, among others.

388

### 389 **Health status and microbiomes.**

390 Much of the work on host-associated microbiomes revolves around whether the community of  
391 microbes in some way affects the health status of the host [86]. In some cases, researchers have  
392 used terms like “*healthy*” or “*dysbiotic*” or “*optimal*” to describe a particular microbiome (*e.g.*,  
393 of one individual at one time) or pattern of change documented among particular groups. While  
394 these terms can sometimes be useful in general discussions of microbiomes, they are hard to  
395 define quantitatively or apply, and therefore more likely to confuse than to illuminate in practice.  
396 For example, an “*optimal*” microbiome could vary between individuals and across environmental  
397 conditions. Similarly, there could be numerous and equivalent alternative stable states, each of  
398 which could be referred to as a “*healthy*” microbiome, and which include transient or permanent  
399 neutral members and/or active symbiotic players. The inverse of a healthy association is  
400 “*dysbiosis*”, often suggested to be any change in the composition and or variability of a microbial  
401 community that can cause any negative impact on the host. Generally, we believe such terms  
402 need to be used with extreme caution (see a useful discussion of this topic in [93]).

403

404 **Ecosystem functions.**

405 Ecosystem functions or processes are generally considered to be aggregate fluxes of energy or  
406 materials [13]. Ecosystem function has also sometimes been defined as the joint effects of all  
407 processes, including fluxes of energy and chemical compounds, that sustain an ecosystem over  
408 time and space through biological activities [94]. Generally, ecosystem functioning depends  
409 disproportionately on a small subset of species in the system. These include particularly  
410 foundation species, dominant sessile invertebrates, plants or algae that provide physical structure  
411 and have a strong role in structuring the community [95]; and keystone species, taxa that have a  
412 large effect on other species that is disproportionate to their own relatively low abundance, and  
413 which if they were removed would drastically change the ecosystem. Resilience is the capacity  
414 of an ecosystem to respond to a perturbation or disturbance by resisting damage and recovering  
415 quickly. Another form of response is evolutionary adaptation where species change genetically  
416 to adapt to a new environment. Over several generations, and through the process of natural  
417 selection, physical and behavioral features of organisms may adapt to function better in the new  
418 environment. If hosts and their associated microbes change in concert, this is termed coevolution.  
419 Moreover, when two co-evolving organisms also undergo speciation, this can lead to the  
420 formation of new species; *i.e.*, co-speciation and co-diversification.

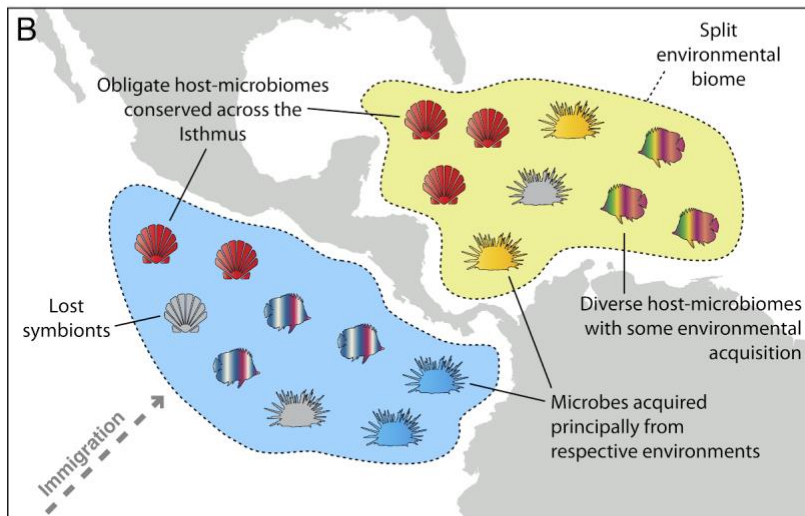
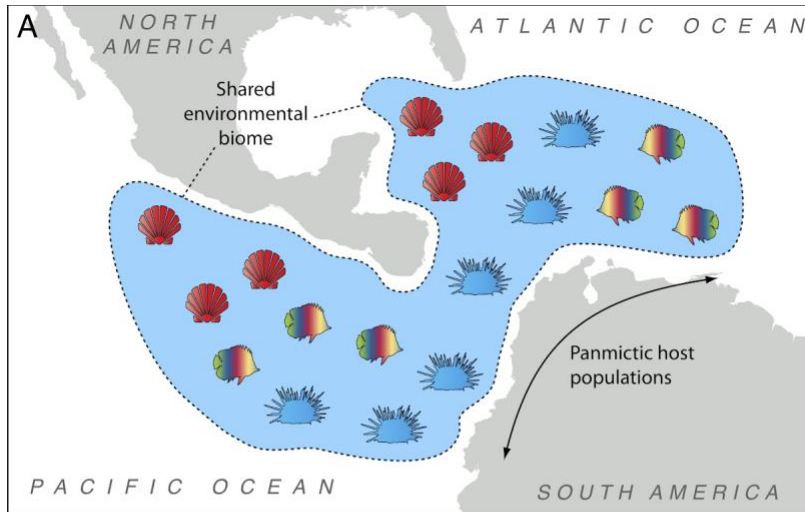
421

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423

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431 **Figure 1. Formation of the Isthmus of Panama split two oceans creating a natural**  
432 **experiment to explore general processes of host-microbe evolution.** (A) Panmictic  
433 populations of hosts and their microbiomes living under similar environmental conditions  
434 became (B) physically isolated when the land bridge formed between North and South America.  
435 Well-calibrated phylogenetic data are available for marine animal hosts such as clams, fishes,  
436 porcelain crabs, snapping shrimps, and urchins. (C) Example of sister species isolated by the  
437 Isthmus of Panama (left: Eastern Pacific, right: Caribbean): fish, *Abudefduf saxatilis* and  
438 *Abudefduf troschelii*; clams, *Ctena mexicana* and *Ctena distinguenda*; and shrimps, *Alpheus*  
439 *panamensis* and *Alpheus formosus*. Photo credit: fish, Ross Robertson (*A. saxatilis*) and A.  
440 *troschelii* from Wikimedia Commons, Hectonichus [CC BY-SA 3.0  
441 (<https://creativecommons.org/licenses/by-sa/3.0>)]; clams, Laetitia G.E Wilkins and Benedict  
442 Yuen; shrimps, Arthur Anker.



443



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