

stazione zoologica anton dohrn

# Marine Microbial Symbioses

...or the power of intimate relationships

Dr. Ulisse Cardini – [ulisse.cardini@szn.it](mailto:ulisse.cardini@szn.it)  
 Head of the Marine Symbiomes Research Group – [www.marinesymbiomes.eu](http://www.marinesymbiomes.eu)

**This lecture covers beneficial host-microbe symbioses found in marine ecosystems.**  
 The goal of this course is to provide students with an understanding of how these diverse and sometimes surprising symbioses have important biological, ecological and human health impacts. The class will begin with a brief introduction to microbial symbiosis before focusing on specific symbiotic interactions and the experimental methods used to discover and characterize them.

Lecturer: **Dr. Ulisse Cardini, PhD**

Head of the Marine Symbiomes Research Group

Integrative Marine Ecology Department, Stazione Zoologica Anton Dohrn

National Institute of Marine Biology, Ecology and Biotechnology

Villa Comunale, 80121 Napoli, Italy

tel: +39 081 5833243

[www.marinesymbiomes.eu](http://www.marinesymbiomes.eu)

[www.szn.it](http://www.szn.it)

Associate Editor of Frontiers in Ecology and Evolution - Section Coevolution

<https://www.frontiersin.org/journals/ecology-and-evolution/sections/coevolution>

Topic Editor of Water

<https://www.mdpi.com/journal/water>



## What's (partly) covered in this lecture

1. An introduction to microbial symbiosis
  - Some history
  - The endosymbiotic theory
  - The holobiont and the hologenome
  - Other key concepts
2. Some examples of marine microbial symbioses
  - Squid symbiosis
  - Chemosynthetic symbioses
  - Coral symbioses
  - Sponge symbioses
3. Microbial symbioses and ecosystem functioning

This is an outline of what will be covered in this 1.5h lecture, but please bear in mind that courses on microbial symbioses usually last 30+ hours, and this lecture will thus barely scratch the surface.

I will first introduce key concepts and definitions in the field of microbial symbioses, and then will move to describe some relevant examples of marine microbial symbioses.

Finally, I will briefly describe why microbial symbioses can be relevant for ecosystem processes.

At the end of this presentation, I will provide some useful resources and references that were used to build this lecture.

If you have any questions, please do not hesitate to interrupt me.



## The origin of the term "symbiosis"

SYMBIOSIS: compound word from ancient greek words σύν  
«together» and βιόω «living»

PARASITISM, MUTUALISM, LICHENISM,  
ETC.. ARE EACH SPECIAL CASES OF THAT  
ONE GENERAL ASSOCIATION FOR WHICH  
THE TERM SYMBIOSIS IS PROPOSED AS  
THE COLLECTIVE NAME.

- HEINRICH ANTON DE BARY -



Studying lichens, in 1879 Heinrich Anton de Bary, a German botanist and microbiologist, defined symbiosis as "the living together of unlike organisms".

He therefore defined symbiosis in the most general sense, encompassing all types of associations.



## Symbiosis *sensu* "De Bary"

INTERACTION	TYPE OF Symbiosis	EXAMPLE
Benefits      Benefits	<b>Mutualism</b> Species A benefits Species B benefits	Sea anemone      Clown fish
Benefits      Unaffected	<b>Commensalism</b> Species A benefits Species B unaffected	Whale      Barnacle
Benefits      Harmed	<b>Parasitism</b> Species A benefits Species B harmed	Dog      Tick

According to De Bary, symbioses are not only mutualistic symbioses, where both partners benefit from the associations, but also neutral or antagonistic interactions such as commensalism and parasitism.



## Symbiosis as any association

### Advantages

- Broad context for research into symbioses
- Reminder to study both costs and benefits
- Reminder that mutualistic and antagonistic processes can be similar

### Disadvantages

- Definition not accepted by most general biologists → it fails to communicate effectively
- It is too general and thus ineffective



The definition of De Bary, including any association, has some advantages,

-because it promotes a broad context for research into symbioses

-it reminds that it is important to study both costs and benefits

-it conveys that the processes underlying relationships that are classified as mutualistic and antagonistic can be similar

However, this definition has also relevant disadvantages as

-the definition is not accepted by most general biologists or non-biologists today, and so it fails to communicate effectively

-there are few principles generally applicable to symbioses, as defined by de Bary, but inapplicable to other biological systems



## Symbiosis as mutualism

The definition of symbiosis we will use here:

An association between different species from which all participating organisms benefit

Which is the benefit?

It depends!

For how long does the association last?

It depends!



The definition of symbiosis which will be used in this lecture, and that is also widely accepted among general biologists is:

an association between different species from which all participating organisms benefit.

From this definition some questions arise, such as:

Which is the benefit? The answer is: It depends! Many associations are complex and variable. If, through a change in environmental circumstance or other factors, the relationship becomes antagonistic, then it is no longer a symbiosis.

For how long should the association last? Again, the answer is: It depends! ...at least a substantial proportion of the lifespan of the interacting organisms.

We shall see more about these questions in the following slides.



## Misunderstandings

Mutually beneficial symbioses were treated as curiosities of nature throughout the 20<sup>th</sup> century

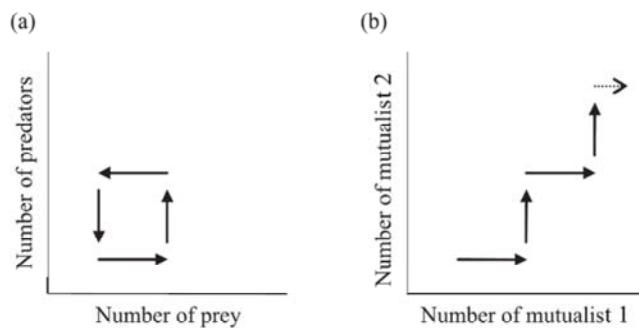


Figure 1-6 Projected population size of interacting organisms in (a) an antagonistic interaction such as a predator-prey relationship and (b) interaction between populations of two mutualists, here labeled as 1 and 2.

Mutually beneficial symbioses were treated as curiosities of nature that were ecologically unstable and evolutionarily transient throughout the greater part of the 20<sup>th</sup> century, because

-by predator-prey (Lotka-Volterra) equations, mutually beneficial interactions lead to uncontrolled population increase while antagonistic interactions tend to stabilize populations

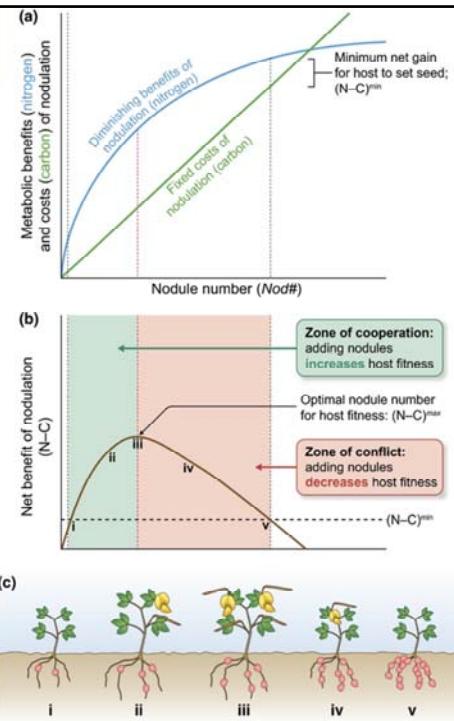
-altruistic genotypes are at a selective disadvantage relative to selfish genotypes



## Conflict vs? symbiosis

These arguments are based on the erroneous assumption that symbioses are perfectly mutualistic

In reality, the partners in symbioses are often in conflict, but the conflict is managed and controlled



Both these arguments are wrong and based on the erroneous assumption that symbioses are perfectly mutualistic. In reality, the partners in symbioses are often in conflict, but the conflict is managed and controlled.

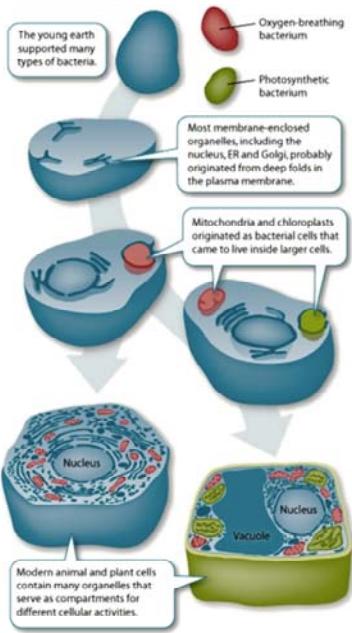
This figure uses the legume-rhizobia symbiosis as an example to model costs vs benefits of the symbiosis. The plant has a cost for nodulation, which is represented by C donated to the symbionts, increasing linearly. The benefit is instead N provided by the symbionts, and increases with a negative exponential function. The net benefit function for nodulation is unimodal, increasing with the formation of nodules (zone of cooperation) until the optimal number of nodules is reached, and above which additional nodules reduce the host benefit (zone of conflict).



## Lynn Margulis and the origin of eukaryotes

“ If you really want to study evolution, you've got go outside sometime, because you'll see symbiosis everywhere!

LYNN MARGULIS



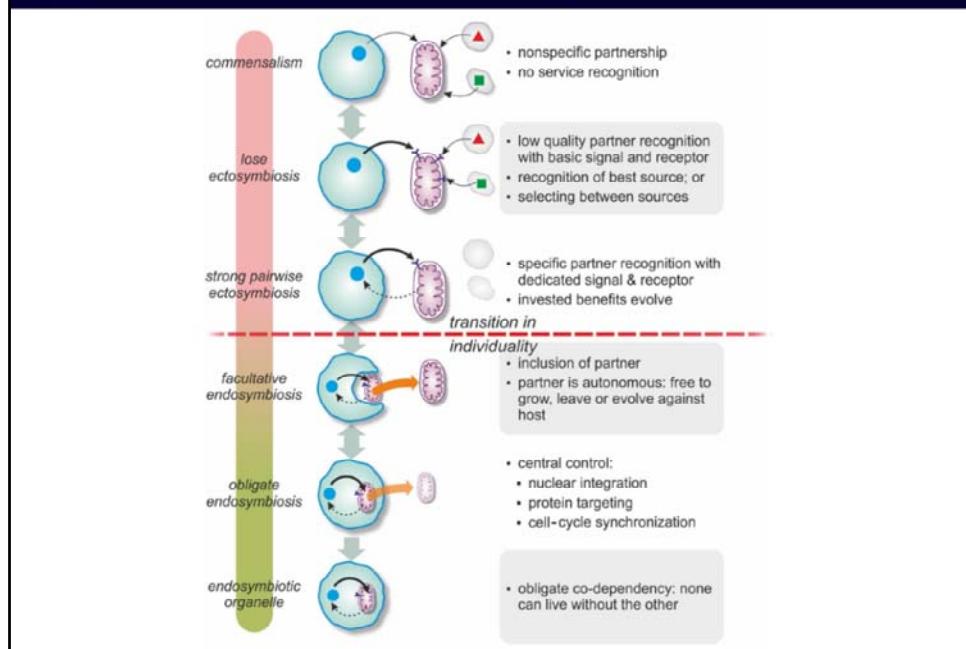
A theory that perfectly illustrates the relevance of mutualistic symbioses in biology is the endosymbiotic theory by Lynn Margulis.

The endosymbiotic theory is the leading evolutionary theory of the origin of eukaryotic cells from prokaryotic organisms, in which mitochondria and chloroplasts (and possibly other organelles of eukaryotic cells) are descended from formerly free-living prokaryotes.

In particular, mitochondria appear to be phylogenetically related to Rickettsiales proteobacteria, and chloroplasts to nitrogen-fixing filamentous cyanobacteria.



## Lynn Margulis and the origin of eukaryotes

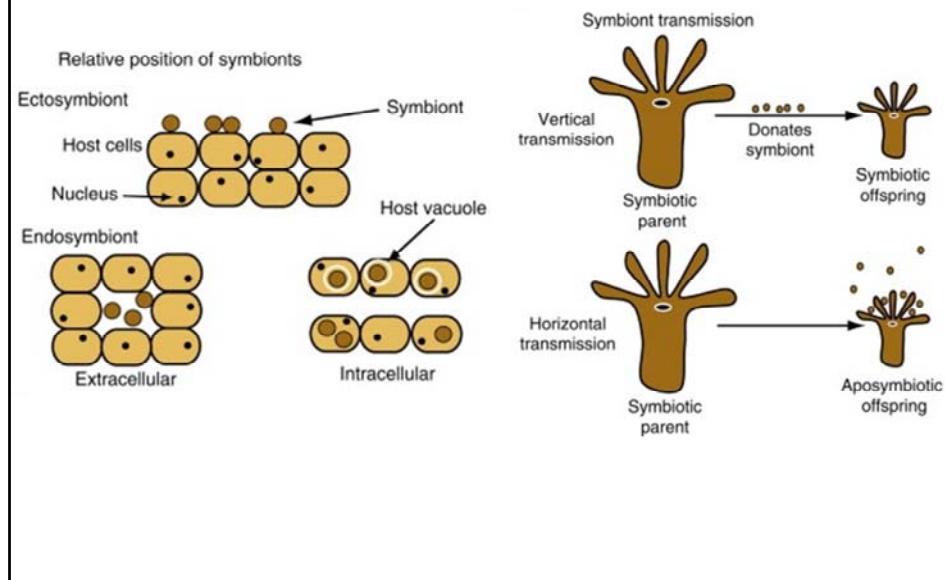


Here we can see the basic steps of endosymbiosis and organellogenesis, from non-specific partnerships all the way to obligate co-dependency, which involves nuclear integration and protein import and it is irreversible.

Solid black arrows represent the source and flow of the various benefits and dashed arrows indicate investments. Colored arrows indicate the option to leave the host.



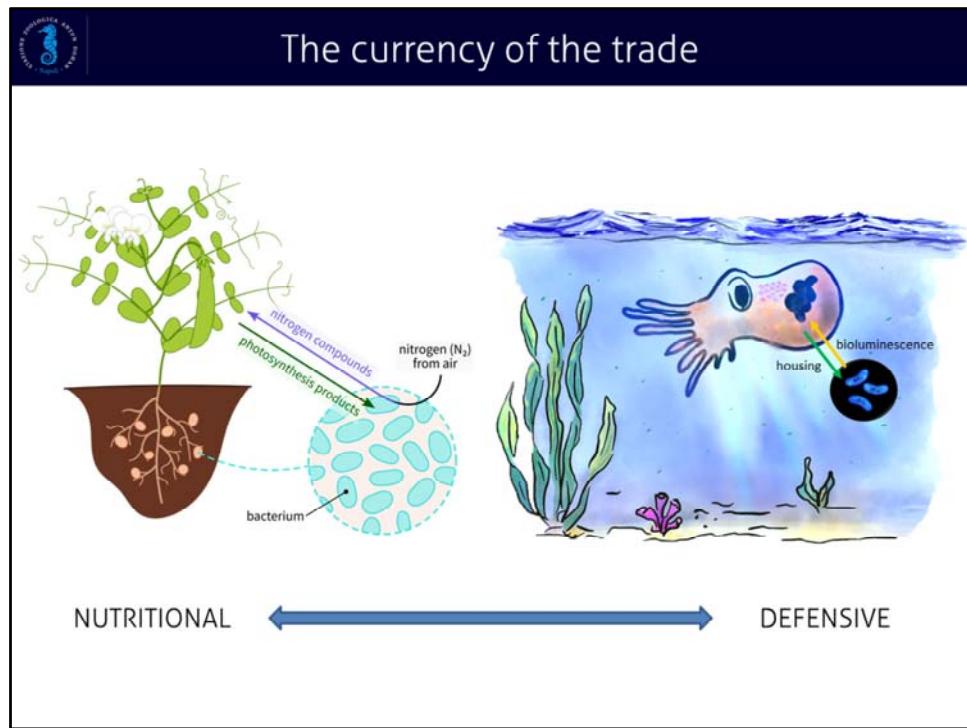
## Positioning and transmission of symbionts



As Lynn Margulis once stated, in the natural world we can see symbiosis everywhere. But of course not all cases reached the integration that was reached in the evolution of mitochondria and chloroplasts.

And thus, we can have examples where the symbionts are ectosymbionts, living on top of the host outer surface, or endosymbionts, when living inside the host body. Endosymbionts can be found either extracellular or intracellular, hosted in specific host vacuoles.

The transmission mode of symbionts also differs in nature, with examples where the symbiont is acquired vertically, from the parents to the offspring, or horizontally, with aposymbiotic juveniles that acquire their symbionts from the surrounding environment, in their early life stages or throughout their life.

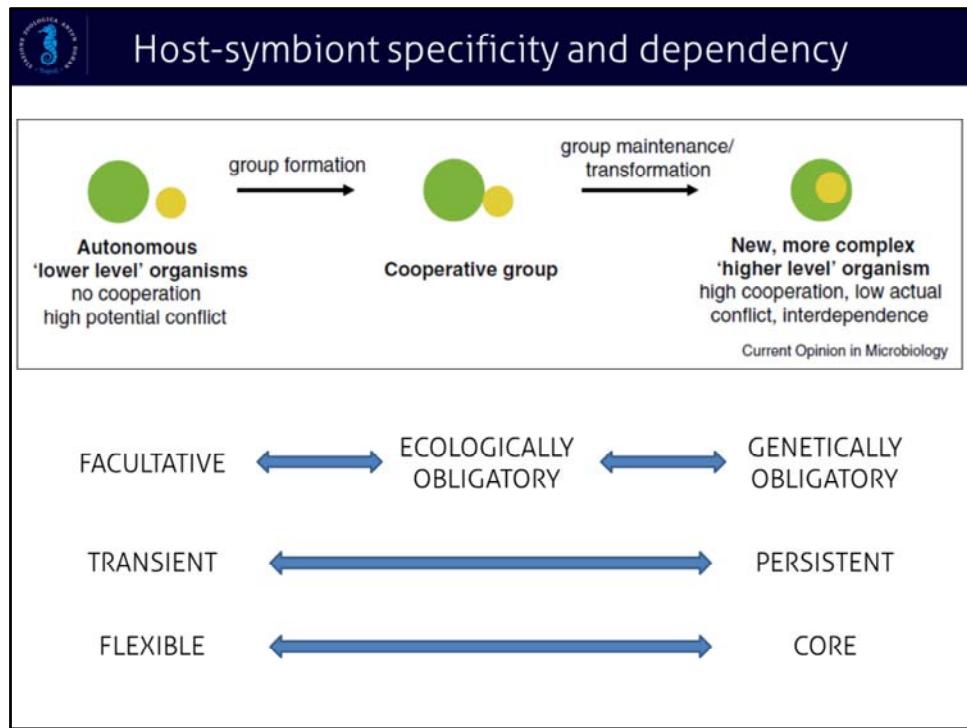


But what's the currency of the symbiosis? Which is the benefit?

This also varies, for example:

The currency in the legume–Rhizobium symbiosis is nutritional. The symbionts provide the host with fixed N<sub>2</sub> in exchange of photosynthetically fixed C.

The currency in the Vibrio-squid symbiosis is defensive. At night, the bacterium helps protect the host by hiding the squid's silhouette from predators using bioluminescence, while the host provides a home to the bacteria, allowing them to multiplying undisturbed.

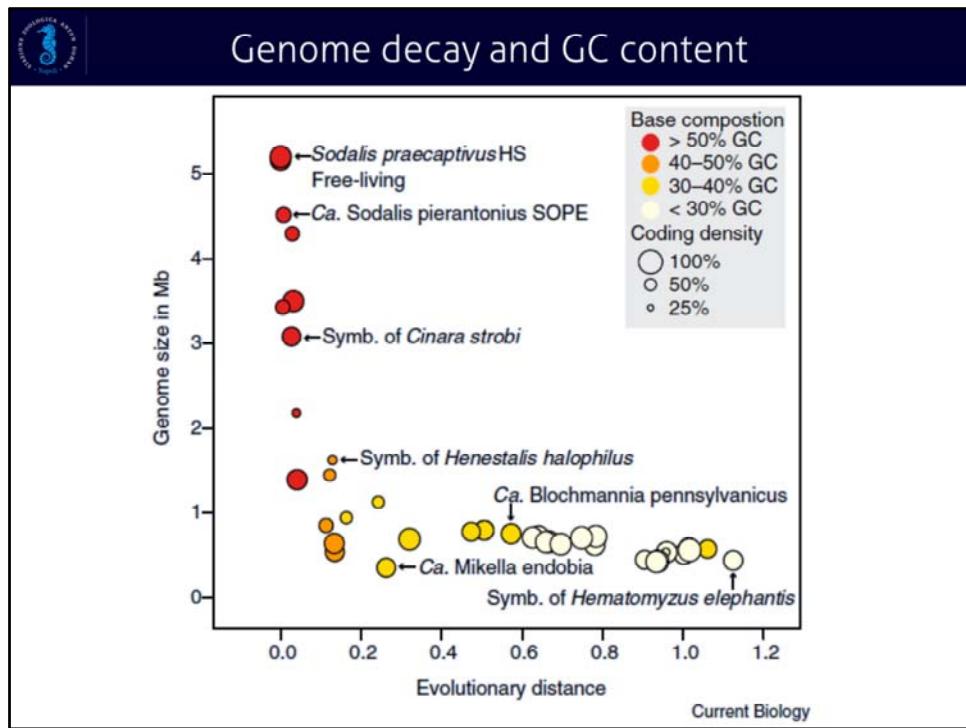


And so again you can see how there is in nature a whole range of integration levels possible between the host and its symbionts.

Here a transition is illustrated between a larger partner (e.g. host) and a smaller partner (e.g. symbiont).

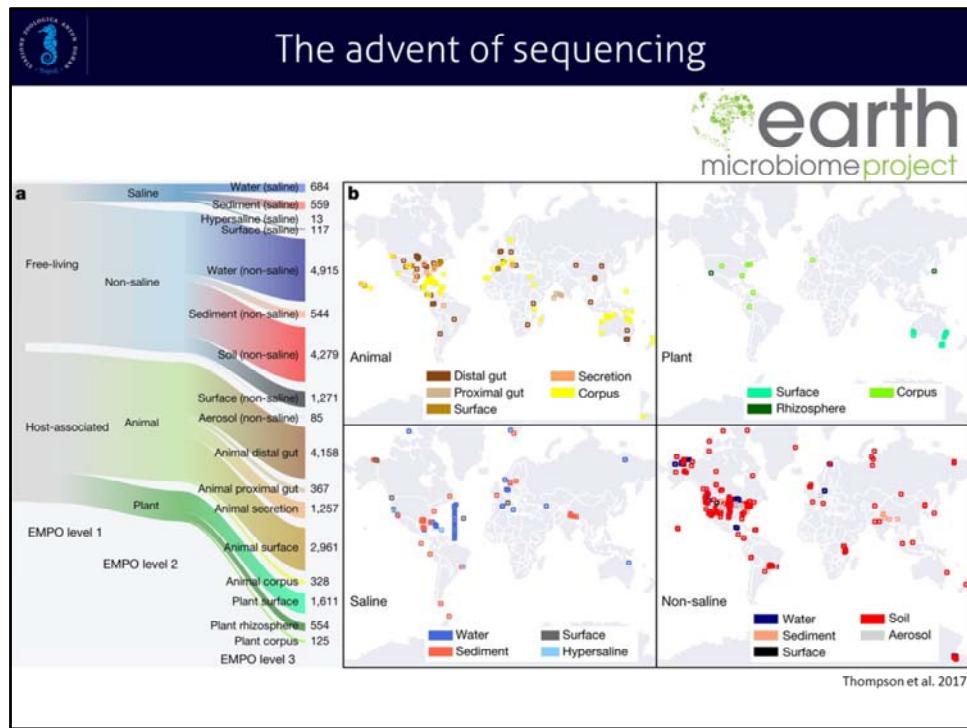
While initially autonomous entities with high potential conflict and low cooperation, the two species ultimately form a symbiotic organism with high cooperation and low conflict, as well as a high degree of mutual dependency.

Other terms that are related to this transition, and refer to the symbionts, are facultative vs ecologically or genetically obligatory; transient vs persistent, flexible vs core.



With increasing integration, following symbiosis in evolutionary time, we often see a reduction in genome size and in GC content in the symbiont, here illustrated for *Sodalis*-related endosymbiotic bacteria (Gammaproteobacteria, Enterobacterales) that associate with insects.

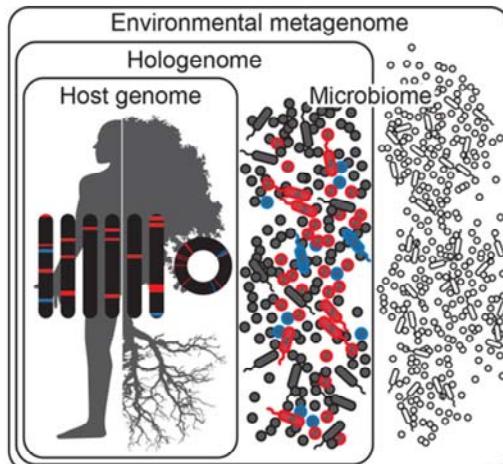
The initial steep decrease shows that genome reduction is rapid relative to sequence divergence at the onset of symbiosis. The trend towards A+T genomic bias can be seen by tracing the dark red points at the top left to the yellow points on the bottom right.



If initial studies were only focused on intimate (mostly endo-) symbioses, with the advent of sequencing and deep sequencing it became clear that microbes are associated with virtually all animals and plants on Earth.



## The holobiont and the hologenome



Theis et al. 2016

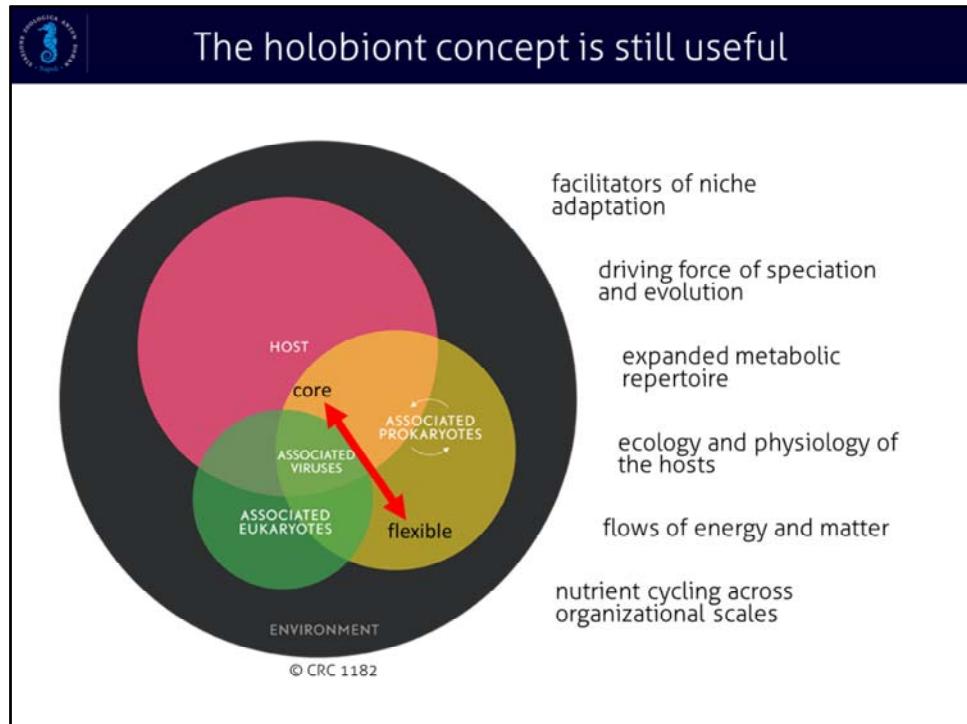
- Host and symbiont genes that alone and/or together affect a holobiont phenotype
- Coevolved host and symbiont genes that affect a holobiont phenotype
- Host genes and symbionts that do not affect a holobiont phenotype
- Environmental microbes that are not part of the holobiont

...maybe a step too far...

This new information made clear that we needed a new framework to look at organismal biology. From that, the concept of holobiont came about, describing the assemblage of the host and its associated microbiome formed by eukaryotes, prokaryotes and viruses. Another important distinction is that between the core microbiome, more closely associated with the host and often species specific, and the environmental microbiome, which varies with varying environmental conditions.

The Hologenome then encompasses the genomes of the host and all of its microbes at any given time point, upon which natural selection was suggested to occur.

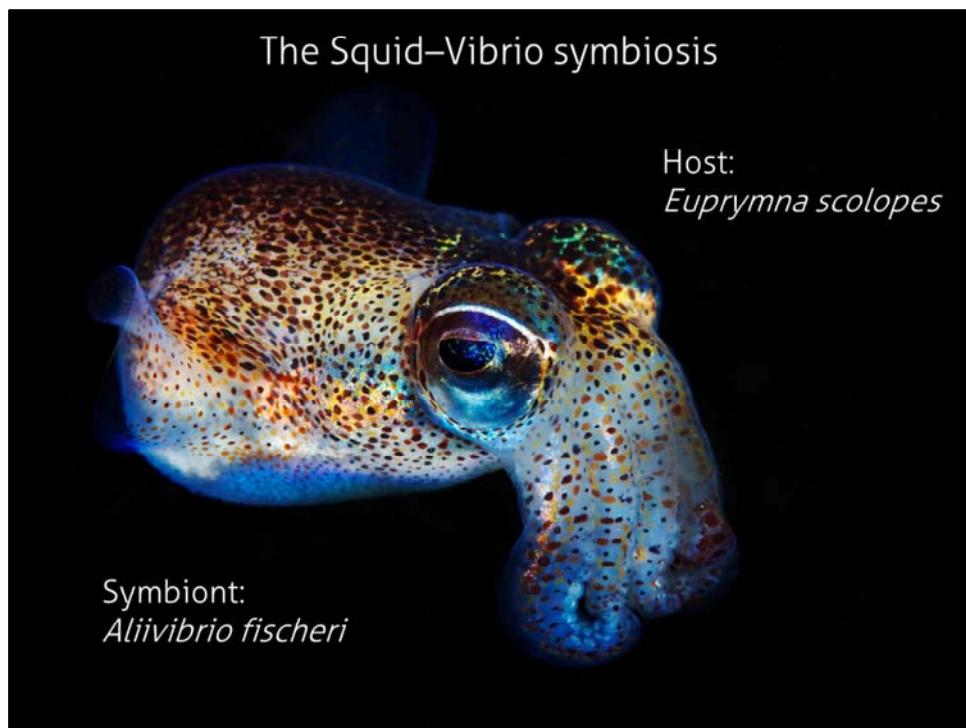
But this is as we saw a step too far, since both level of integration and type of interaction between partners can vary a whole lot within a holobiont.



The term holobiont is still useful, to simply define high-diversity symbioses, as a theoretical and experimental framework to study the interactions between hosts and their associated microbial communities in all types of ecosystems.

The microbial community in a holobiont (or at least some of its members) can act as facilitators of niche adaptations and as driving force of speciation and evolution, and can add an expanded metabolic repertoire to their host.

For this reasons, they strongly influence ecology and physiology of their hosts, they can contribute significantly to flows of energy and matter in and out of the organism, and more generally can affect nutrient cycling across organisational scales.



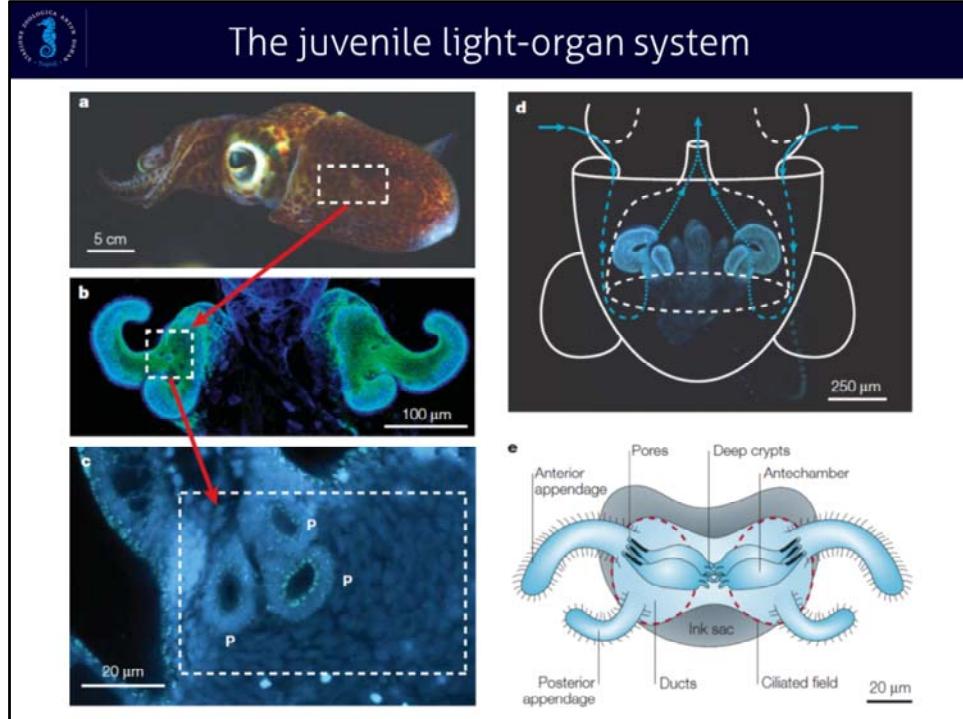
And now let's move on to understand some wonderful examples of marine microbial symbioses.

The first example may also be the cutest one. This is the squid-vibrio symbiosis.

The host is the squid *Euprymna scolopes*, a small marine invertebrate that spends its nights hunting for prey in shallow waters of Hawaii.

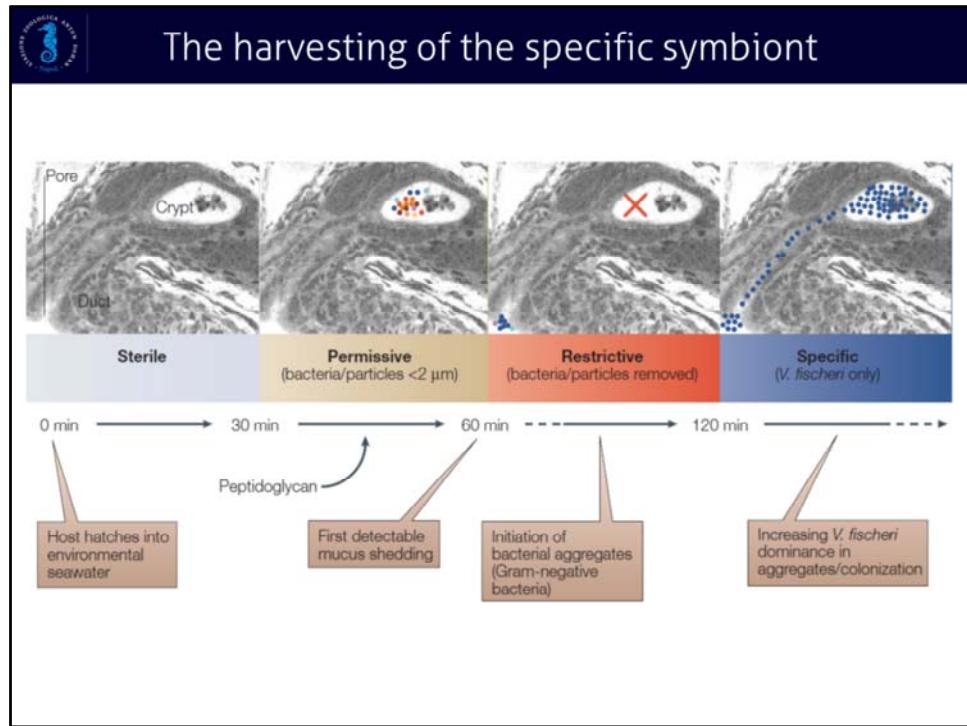
The microbial symbiont is the Gram-negative rod-shaped gammaproteobacterium *Aliivibrio fischeri*.

It is a marine heterotroph belonging to the family Vibrionaceae found throughout the world in temperate and subtropical regions, existing as either a free-living saprophyte, a member of the microbial gut community in many marine mammals, or a light organ symbiont in several species of squid and fish.



The juvenile squid hatches into seawater teeming with microorganisms (1 million cells per ml of seawater!), and although *V. fischeri* cells make up less than 0.1% of the natural seawater bacterial community, the light organ becomes colonized within hours after the squid hatches, with the squid able to select from different *V. fischeri* strains (!!!) to find the most suitable symbionts.

Here you can see the ciliated epithelium on the surface of the juvenile light organ (b), with its pores through which the symbionts enter host tissues (c). In (d) you see how the water flows through the mantle cavity and in (e) the internal components of the squid light organ at hatching.



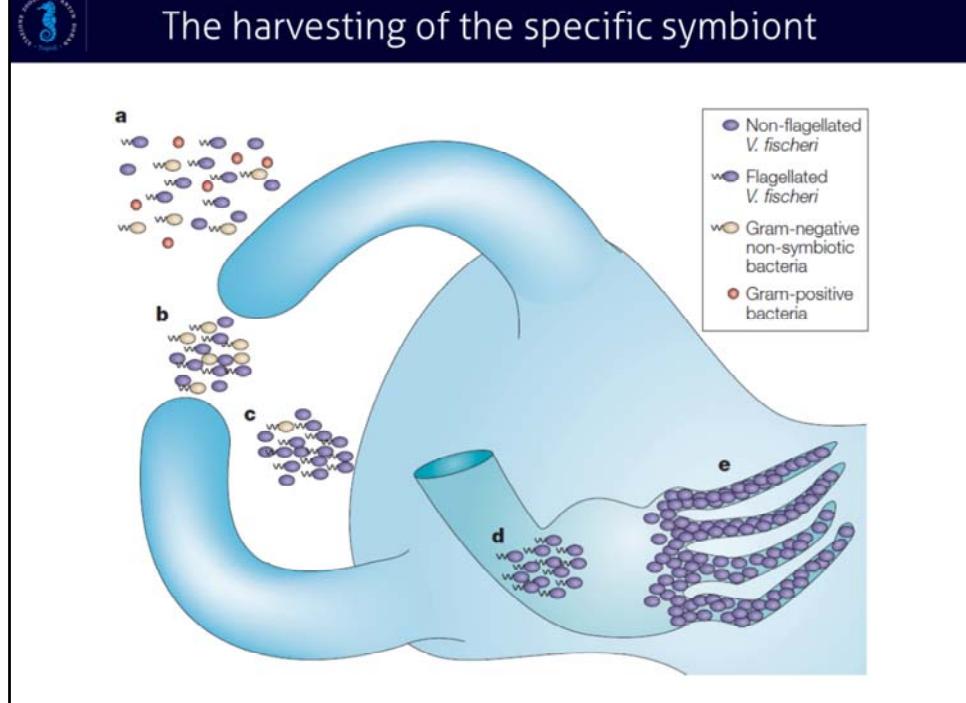
Between 30 and 60 minutes after hatching, the light-organ crypts are open to small numbers of bacterial cells or particles less than 2  $\mu\text{m}$  in diameter, which are later removed by an as-yet-unknown mechanism.

After 1 hour, the host sheds mucus in response to bacterial peptidoglycan and by 2 hours, *V. fischeri* (~1  $\mu\text{m}$  in diameter) begin to aggregate above the pore and then migrate through the duct before colonizing the crypt epithelium.

At this point, the light organ transforms from a ‘permissive’ environment to an environment that is exclusive to the symbiont.

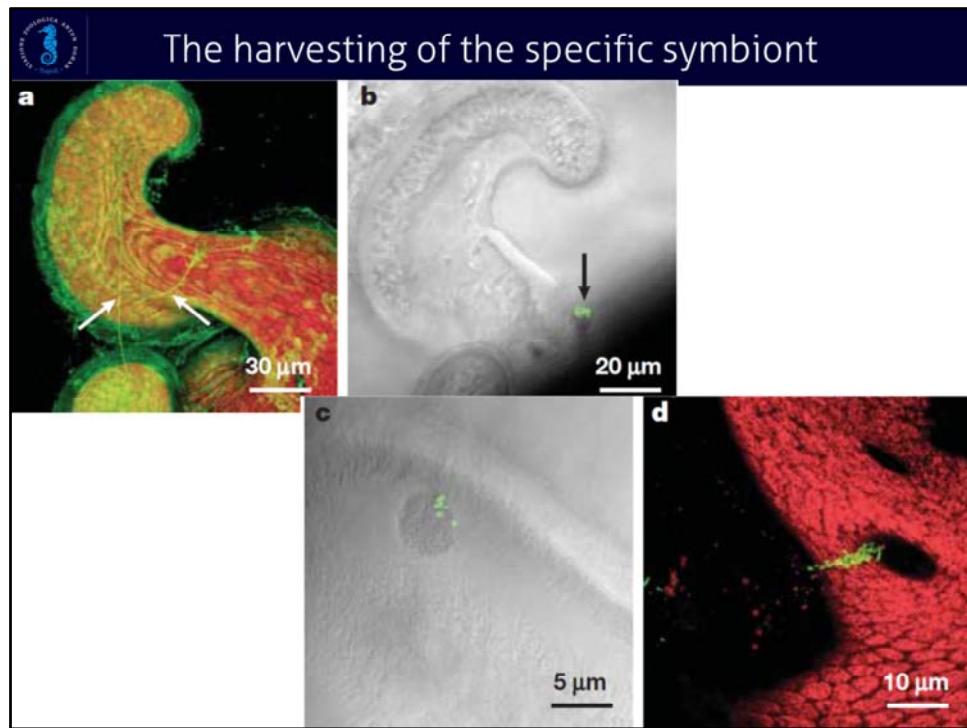


## The harvesting of the specific symbiont



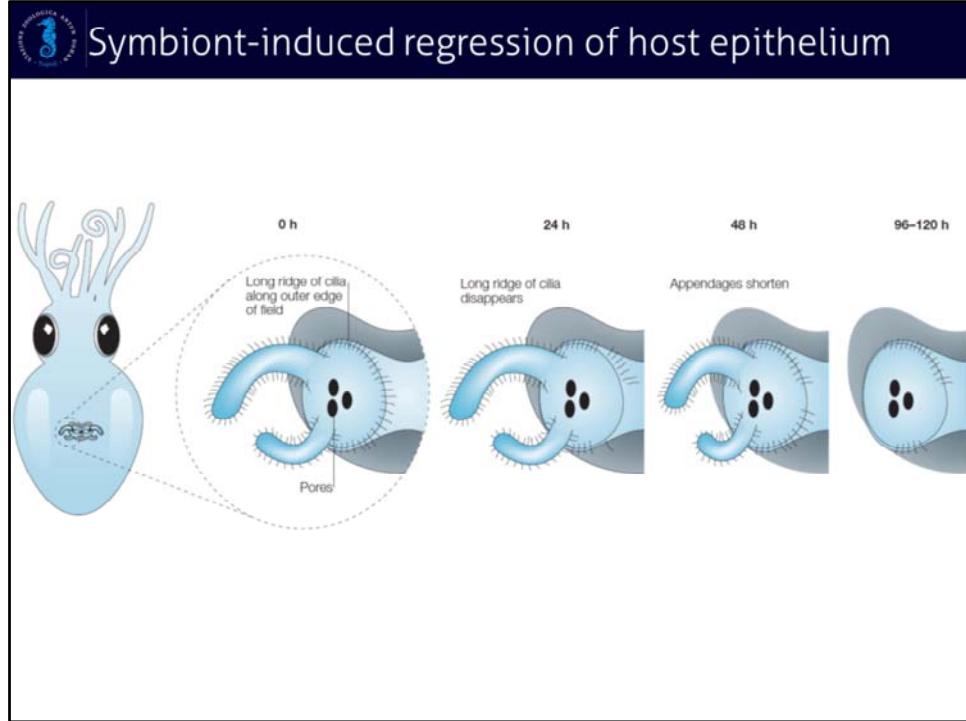
This model depicts the progression of light-organ colonization as a series of steps, each more specific for symbiosis-competent *Vibrio fischeri*.

- In response to Gram-positive and Gram-negative bacteria (alive or dead) the bacterial peptidoglycan signal causes the cells of the ciliated surface epithelium to secrete mucus.
- Only viable Gram-negative bacteria form dense aggregations.
- Motile or non-motile *V. fischeri* out-compete other Gram-negative bacteria for space and become dominant in the aggregations.
- Viable and motile *V. fischeri* are the only bacteria that are able to migrate through the pores and into the ducts to colonize host tissue.
- Following successful colonization, symbiotic bacterial cells become non-motile and induce host epithelial cell swelling. Only bioluminescent *V. fischeri* will sustain long-term colonization of the crypt epithelium.



Here you can see again the harvesting of the specific symbiont, in

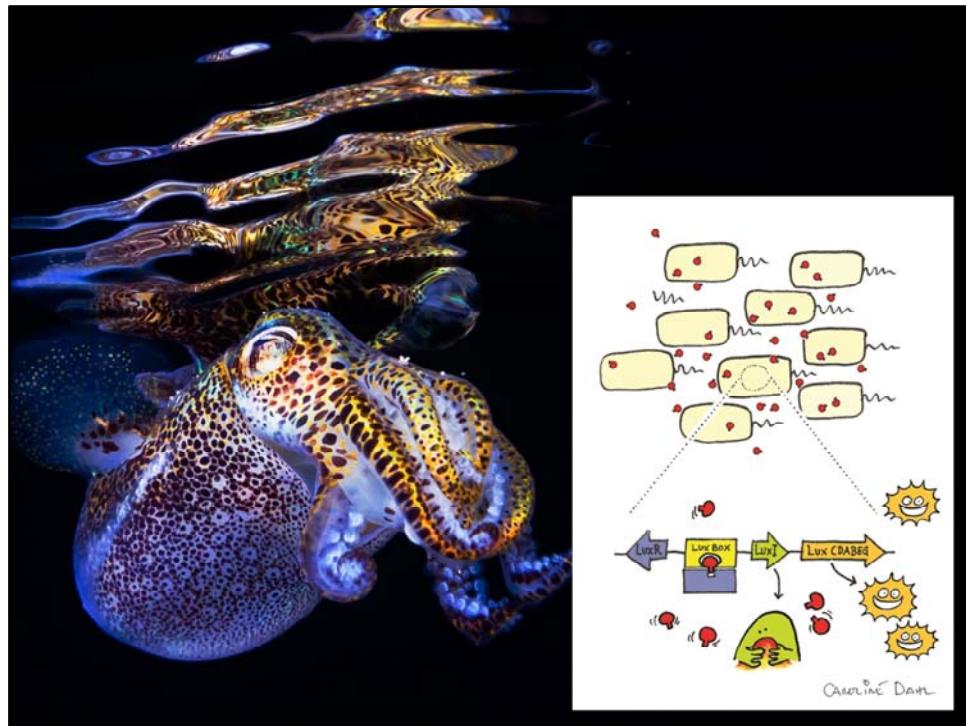
- a ) In response to bacterial peptidoglycan, the cells of the ciliated superficial epithelium begin to secrete mucus (indicated by arrows).
- b) This mucus is used as a substrate in which *Vibrio fischeri* (green) will form aggregations (indicated by an arrow).
- c) Although many viable Gram-negative bacteria will aggregate, *V. fischeri* outcompetes other bacteria for space in the aggregations. Shown is a *V. fischeri*-dominated aggregation with non-symbiotic *V. parahaemolyticus* (green).
- d) After aggregating for 2–5 hours, *V. fischeri* (green) migrates through the pores and ducts and colonizes the host tissue.



Once colonization has taken place, the symbiotic *V. fischeri* cells in the crypts trigger a series of developmental events that transform the light organ from an instrument of symbiont acquisition to the functional adult light organ.

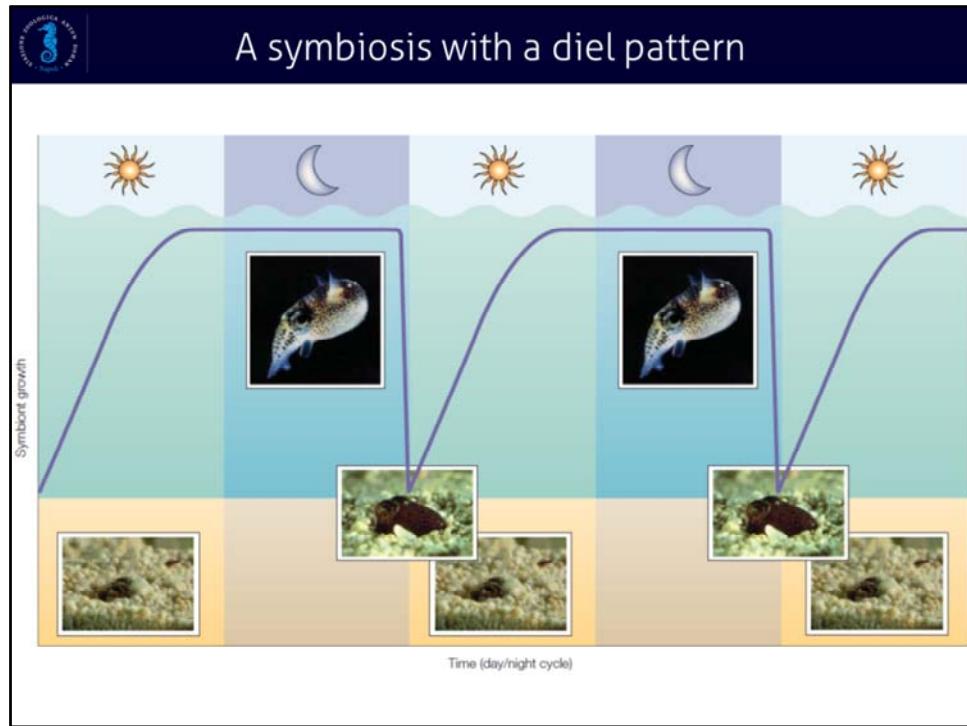
Here you can see the gradual, symbiont-induced regression of the ciliated epithelium of the juvenile light organ after successful colonization.

*Vibrio fischeri* induces widespread programmed cell death (apoptosis) and the complete regression of this 'symbiont-harvesting apparatus' over a period of 4 to 5 days.



And so, finally, the adult squid can go out at night, protected by its army of symbiotic bacteria. In response to signals coming from a light-sensing organ of the animal host detecting moonlight, the symbiotic *V. fisheri* will produce the right amount of light through the LUX operon and quorum sensing.

But how is symbiont numbers controlled by the host?



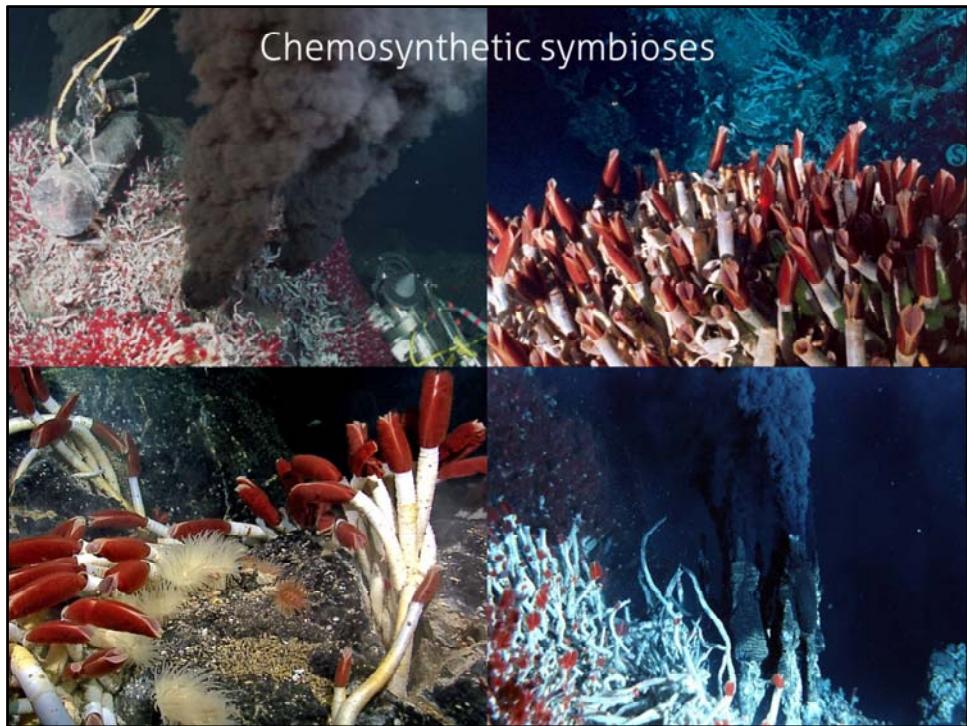
The squid *Euprymna scolopes* is nocturnal and emerges at dusk to hunt.

During this period the light organ is full of *Vibrio fischeri* and the bioluminescence from this bacterium is used by the squid to camouflage itself from potential predators.

At dawn the host expels ~95% of the light-organ bacteria into the surrounding environment.

The squid then buries beneath the sand, and during the day the remaining 5% of *V. fischeri* that remain in the light organ grow (indicated by the purple line) such that, by mid-afternoon, the light organ is again full of bacteria.

Not only does this behaviour help maintain the symbiont population in the host, but it also seeds the environment with *V. fischeri* for colonization of the next generation of squid.

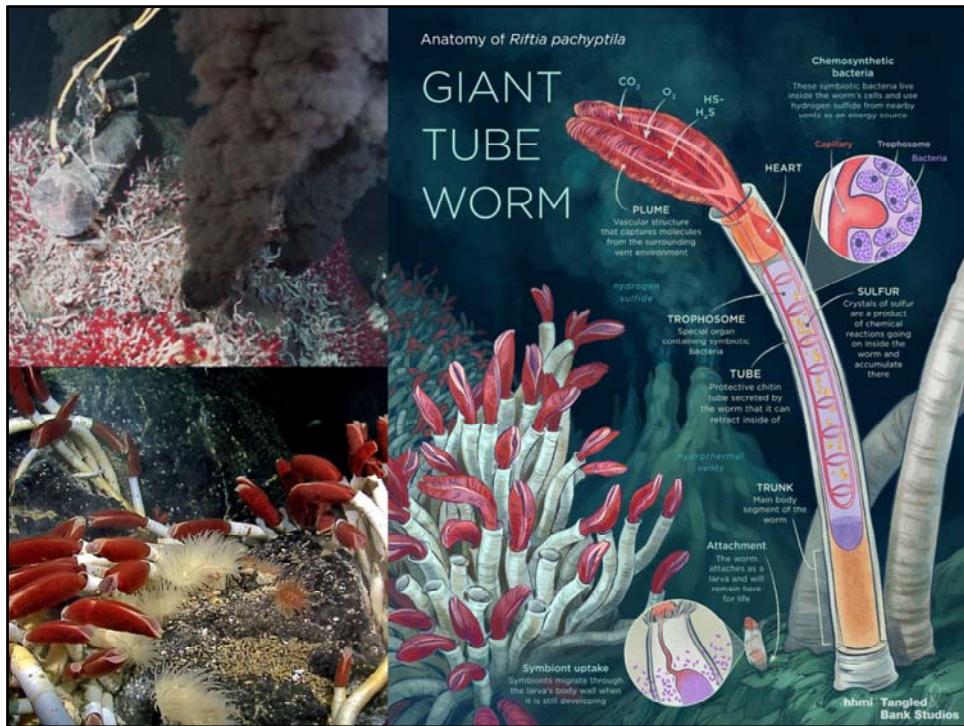


Our second example of marine microbial symbioses is that of chemosynthetic symbioses.

No one knew chemosynthetic symbioses existed until 45 years ago, when teeming communities of animals were found thriving at hydrothermal vents two and a half kilometers below the sea surface.

The discovery of these lightless ecosystems revolutionized our understanding of the energy sources that fuel life on Earth.

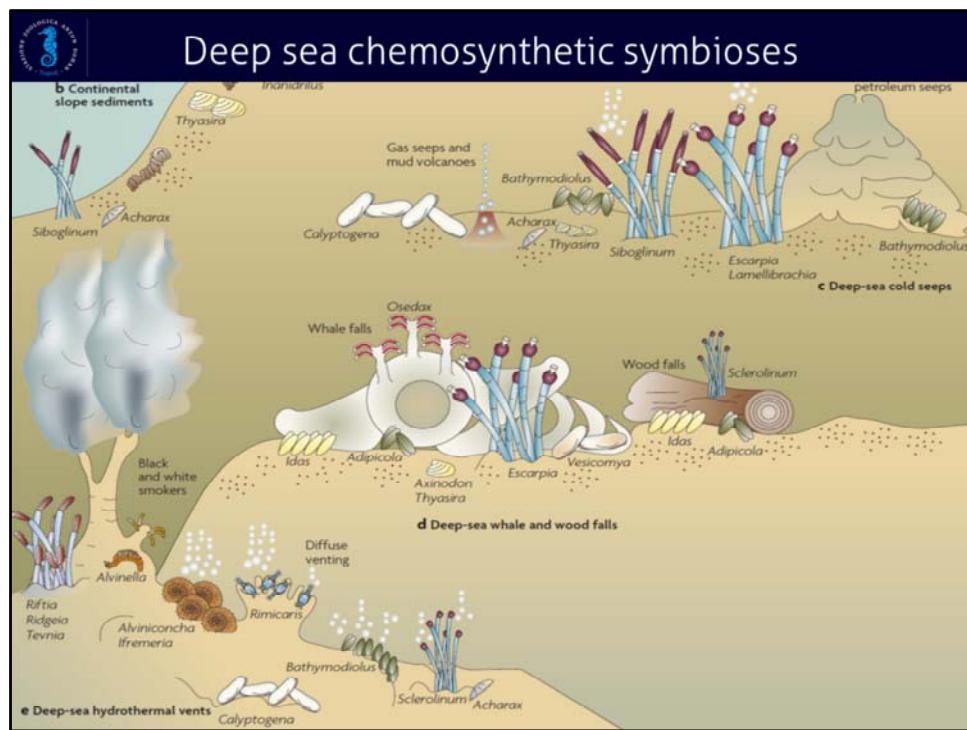
The first of such animal symbioses to be discovered was that of the giant tube worm *Riftia pachyptila*



*R. pachyptila* lives on the floor of the Pacific Ocean near hydrothermal vents, and can tolerate extremely high hydrogen sulfide levels. These worms can reach a length of 3 m and their tubular bodies have a diameter of 4 cm. Ambient temperature in their natural environment ranges from 2 to 30°C.

The upper body region is the vascularized branchial plume, which is bright red due to the presence of a specific hemoglobin that is able to bind both O<sub>2</sub> and H<sub>2</sub>S, which are necessary for both the animal and its chemosynthetic bacteria.

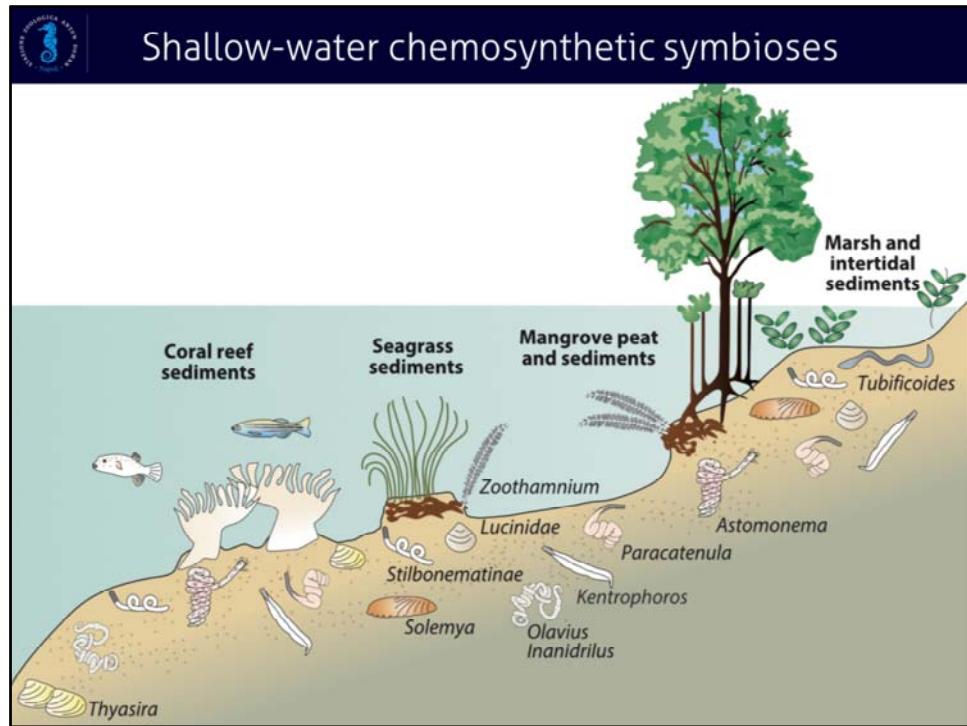
In the upper part of its chitinous tube is located the trophosome, a spongy organ where a billion symbiotic, thioautotrophic bacteria and sulfur granules are found that allow this animal to live in such a challenging deep sea environment.



These and other animals thrive at vents because they live in a nutritional symbiosis with chemosynthetic bacteria that grow on chemical compounds gushing out of the vents, such as sulfide and methane, which animals cannot use on their own.

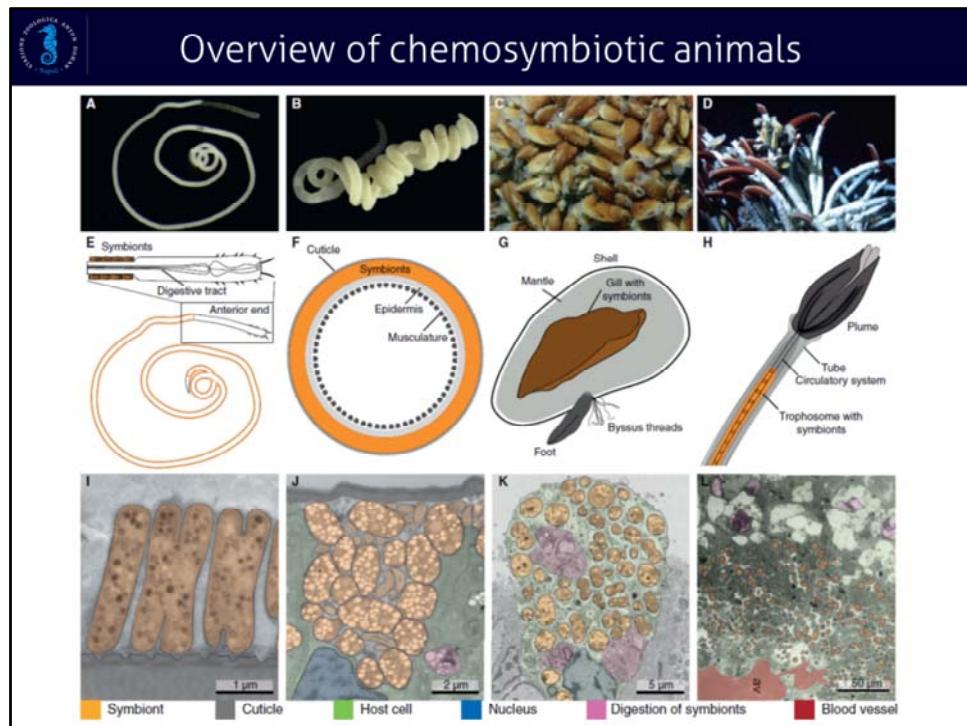
The symbionts gain energy from the oxidation of these reduced substrates to fix CO<sub>2</sub> and other simple carbon compounds into biomass, which is then transferred to the host.

By associating with chemosynthetic bacteria, animals and protists can thrive in environments in which there is not enough organic carbon to support their nutrition, such as the deep sea.



Ironically, chemosynthetic symbioses are abundant in much more easily accessible habitats such as shallow water sediments, but these were only discovered more recently.

Today, we find chemosymbioses flourishing in environments where sulfide and other reduced energy resources co-occur with oxidants such as oxygen and nitrate. For example, we can find them in mangrove and seagrass sediments, but also in coral reef sands.



Chemosynthetic symbioses have established numerous times independently in a broad variety of animal phyla including nematodes, annelids, mollusks, flatworms, sponges and arthropods.

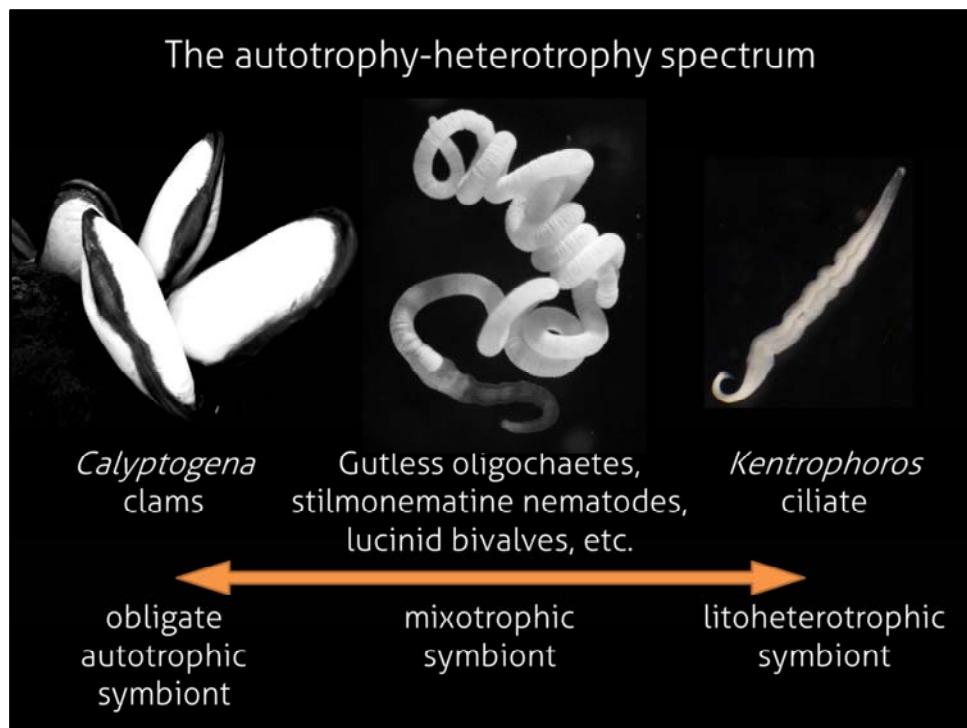
Here you can see different examples of chemosynthetic symbioses, from left to right

The stilbonematine nematode *Laxus oneistus* is coated with a single sulfur-oxidizing symbiont phylotype; sulfur vesicles in these symbionts give the hosts their white coloration. The rod-shaped symbionts divide along their length, allowing both cells to remain attached to the host after cell division.

The gutless oligochaete *Olavius algarvensis* has completely reduced its digestive and excretory systems, and relies on its bacterial symbionts for nutrition and waste removal. The bacteria occur in a thick layer just below the worm's cuticle.

Deep-sea *Bathymodiolus* mussels are common at both hot vents and cold seeps. The mussels harbor their symbionts in their enlarged gills as intracellular symbionts within specialized cells called bacteriocytes.

And finally, the giant tubeworm *Riftia pachyptila*, which we saw before.



The vast majority of chemosynthetic symbionts are thiotrophs that oxidize reduced sulfur compounds to gain energy and reducing equivalents to fuel carbon fixation.

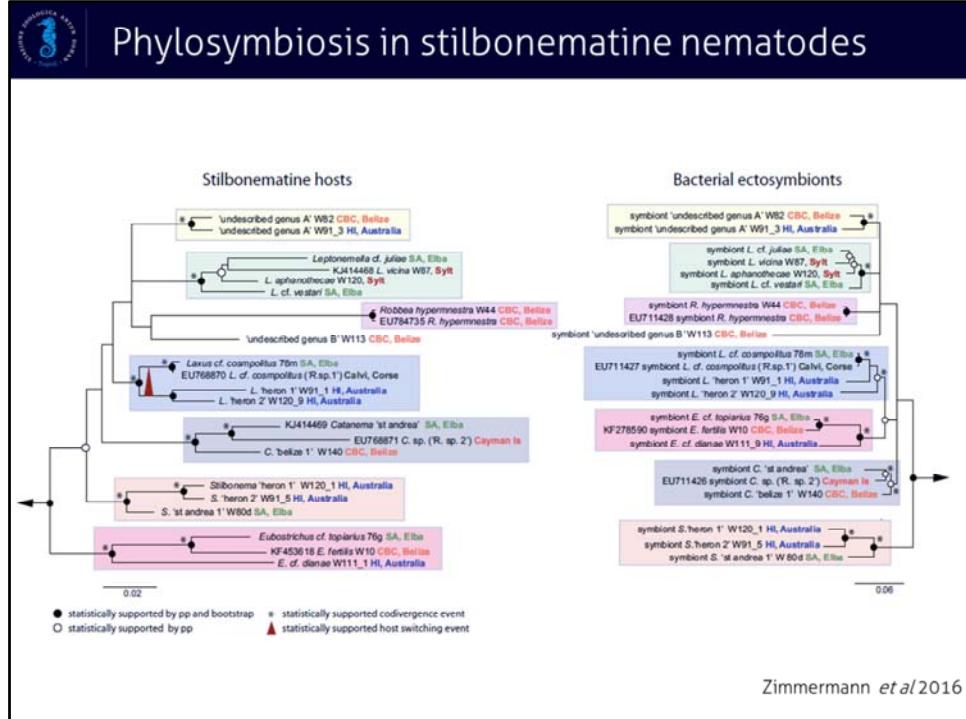
The diversity of metabolisms is however impressive, with many chemosymbionts that are methanotrophs, using methane as an energy as well as a carbon source. Other energy sources include hydrogen and carbon monoxide.

Chemosynthetic symbionts cover the whole spectrum between obligate autotrophy in deep sea clams and obligate heterotrophy in the *Kentrophoros* ciliate, but most of them are mixotrophs.

To this spectrum also correspond a whole range of reproductive strategies and symbiont transmission modes, from vertical to mixed to horizontal, with symbionts acquired from the environment throughout the host life span.



## Phylosymbiosis in stilbonematine nematodes

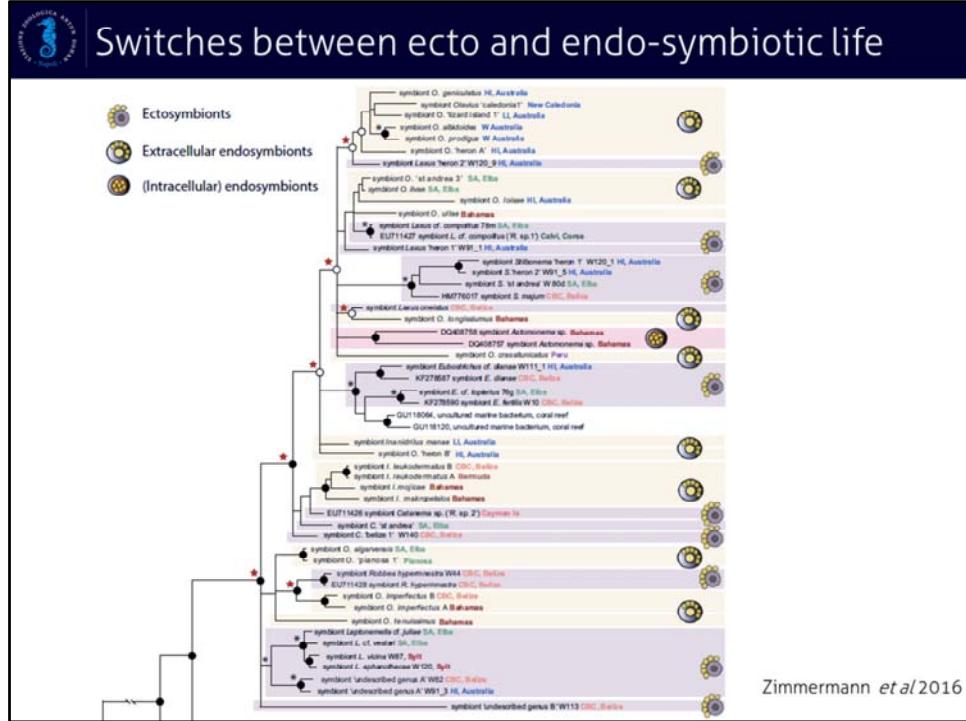


Despite their remarkable symbiont flexibility, however, the symbionts are very loyal to their hosts in some concerns.

For example, in the ectosymbiotic associations of stilbonematine nematodes there is a high degree of congruence between symbiont and host phylogenies, based on their ribosomal RNA (rRNA) genes.

This pronounced codivergence is remarkable given that the symbionts are attached to the surface of their hosts and constantly exposed to the surrounding sediment. Also, stilbonematines moult and shed their cuticle four times during their life cycle as all marine nematodes, providing ample opportunities for symbiont displacement by other free-living bacteria.

The high genus and species specificity as well as evolutionary stability of the stilbonematine ectosymbiosis implies that highly specific mechanisms for recognition and maintenance have evolved to sustain these symbioses!

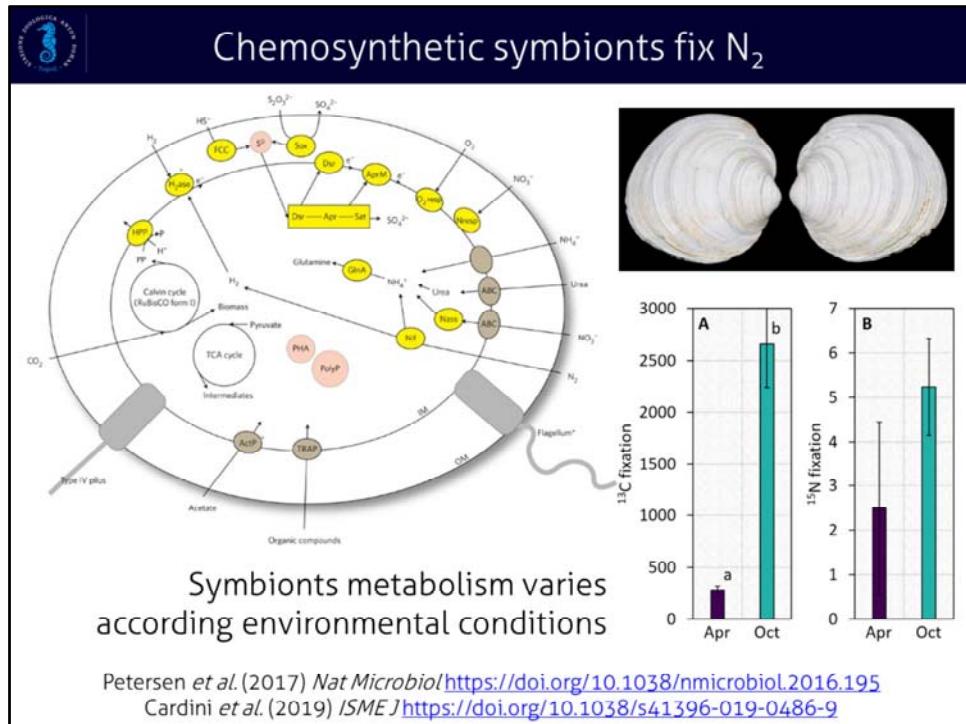


Comparing the phylogenies of *Cand. Thiosymbion* living as ecto- and endosymbiont, this study found remarkably close relationships between these despite the large phylogenetic distances between their three worm host groups (stilbonematine nematodes, *Astomonema* nematodes, and gutless oligochaete worms).

Host switching of *Cand. Thiosymbion* between the two animal phyla Nematoda and Annelida is intriguing as it predicts the ability of the bacterial symbiont to shift between ecto- and endosymbiotic lifestyles.

This can have a twofold explanation:

- 1) Similar function of the symbionts: autotrophic sulphur oxidizers that provide their hosts with nutrition.
- 2) Similar benefits to their symbionts: both hosts migrate between the upper oxidized and the lower reduced layers of the sediment and thus provide their symbionts with access to electron acceptors such as oxygen in the surface layers and electron donors such as reduced sulphur compounds in the lower layers.



In previous work, my colleagues and I could show that not only chemosynthetic symbionts possess a flexible metabolism concerning energy and carbon sources, but are in some instances also capable of fixing atmospheric dinitrogen gas through N<sub>2</sub> fixation.

Our study focused on the symbiont of *Loripes orbiculatus*, a chemosymbiotic clam common throughout the Mediterranean sea. Using metagenomics, metatranscriptomics and metaproteomics, we could show that the symbionts have and express all the molecular machinery for carrying out N<sub>2</sub> fixation.

In a subsequent study, we demonstrated that N<sub>2</sub> fixation actively occurs, but that their metabolism varies with changing environmental conditions along with seasonality. These studies confirmed once more the specificity and flexibility of these symbioses.

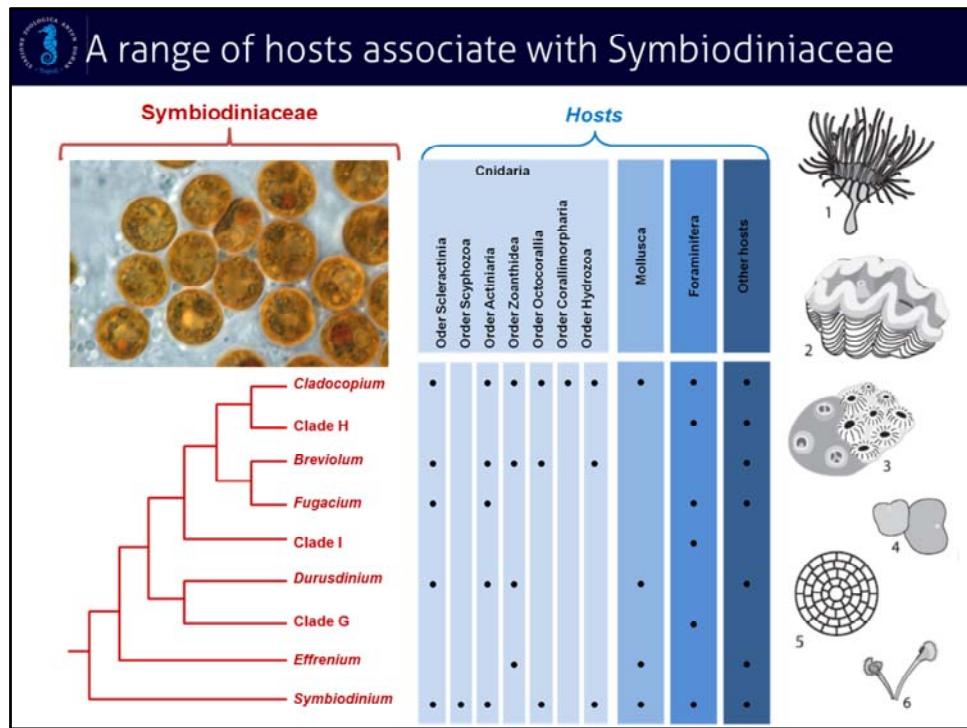


## The coral holobiont



After chemosynthetic symbioses living in the deep sea or in organic-rich sediments, we turn our eye to the oligotrophic tropical waters where the symbiosis between cnidarians (e.g., corals or sea anemones) and intracellular dinoflagellate algae of the family Symbiodinaceae is of immense ecological importance.

In particular, this symbiosis promotes the growth and survival of reef corals in nutrient-poor tropical waters and indeed, coral reefs could not exist without their symbionts.



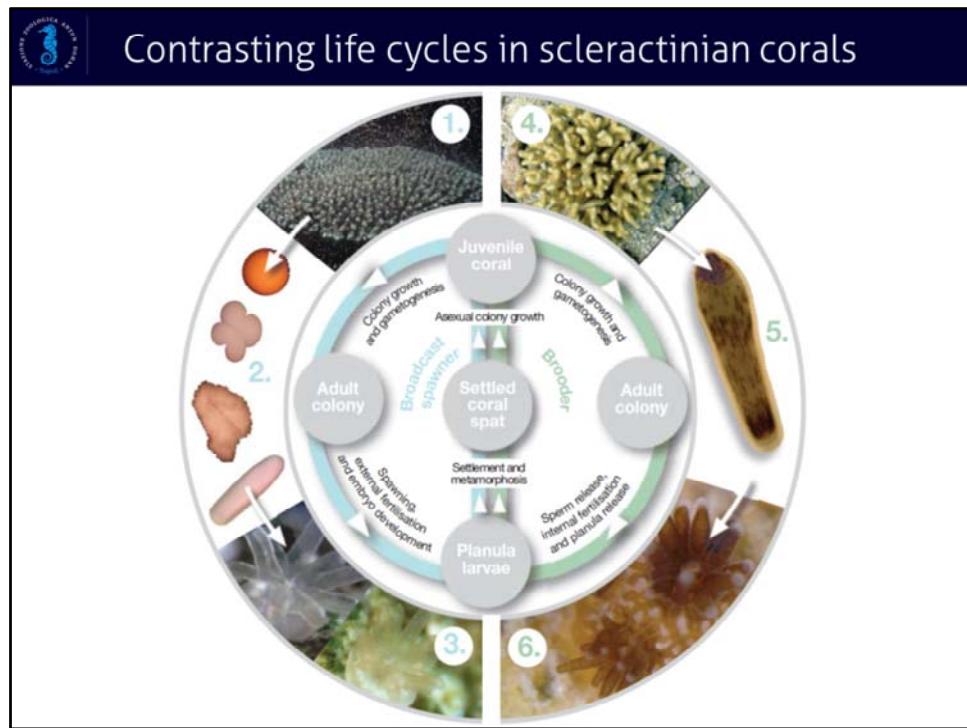
Symbiodinaceae (also called zooxanthellae) is a family of dinoflagellates that encompasses the largest and most prevalent group of endosymbiotic dinoflagellates known.

Symbiodinaceae were historically separated in clades, but molecular-genetic divergence between and within clades recently justified the elevation of most of them to genera, avoiding ambiguity from morphological evidence.

Symbiodinaceae have been identified to establish associations with a variety of host species, such as (1) Cnidaria, (2) Mollusca, (3) Porifera, (4) Platyhelminthes, as well as single-celled eukaryotes such as (5) Foraminifera and (6) Ciliates.

However, specificity and fidelity of relationship over space and time can vary and each host, in a given environment, only associates with a subset of the algae it could potentially harbor.

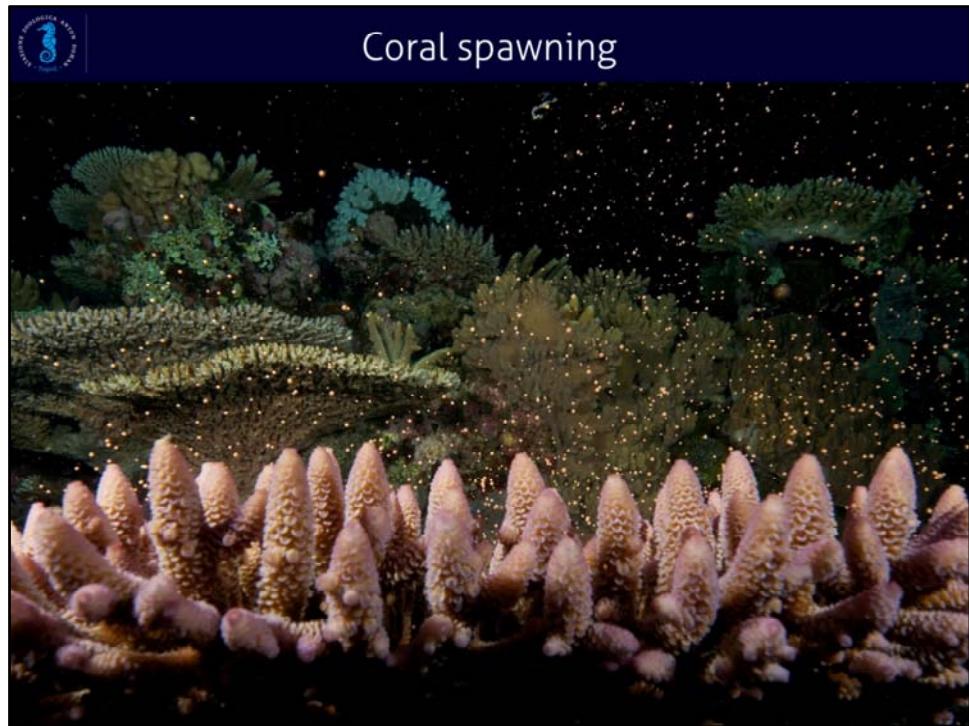
Here, we will focus on the association of Symbiodinaceae with scleractinian corals.



Scleractinian corals differ in their reproductive mode between broadcast spawning and brooding corals.

In broadcasters, on the left, mature colonies spawn many thousands of eggs and sperm at night, and fertilization and larvae development occur in the water column. The larvae then settle on available hard substrata and metamorphose to become azooxanthellate polyps. Soon after the polyp acquires zooxanthellae from the surrounding environment. They thus have a horizontal transmission mode of the symbionts.

In brooders, on the right mature colonies fertilization is internal, and a fully formed planular larvae is released which is already hosting the symbiotic zooxanthellae. They thus have a vertical transmission mode of the symbionts.

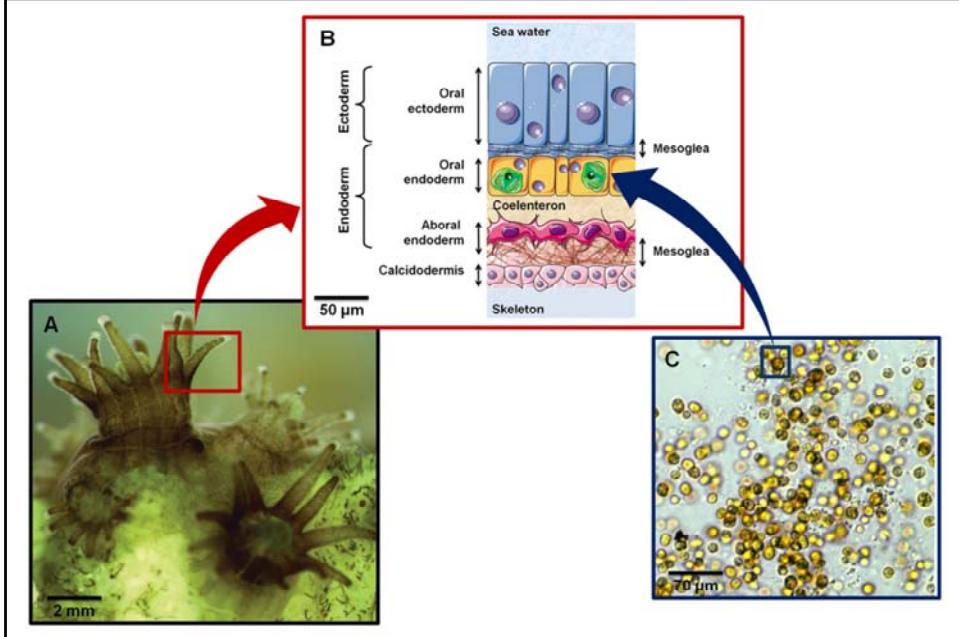


Coral spawning

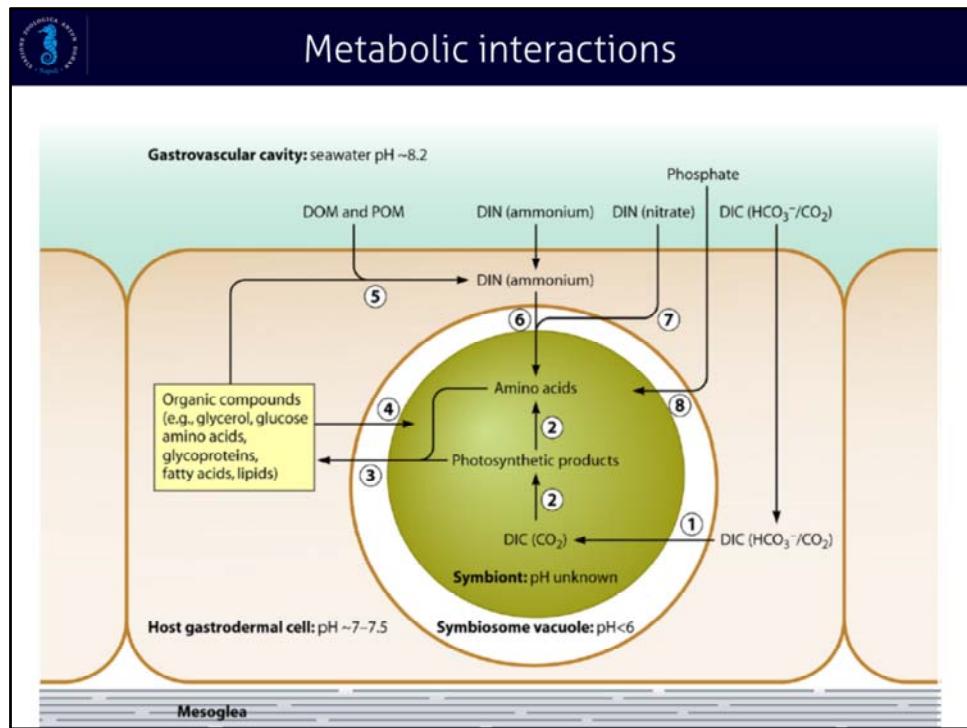
Once a year, on cues from the lunar cycle and the water temperature, broadcast spawning corals release their eggs and sperm all at the same time. This spawning cycle is one of nature's most spectacular events.



## The anatomy of a coral symbiosis



Corals are composed of two cell layers: an ectoderm (epidermis) and an endoderm (gastrodermis). Zooxanthellae (represented as green structures in oral endoderm) are found in vacuolar host membranes (symbiosome) in the gastrodermal cells. Yellow-brown color is due to characteristic dinoflagellate pigments (diadinoxanthin, peridinin) as well as chlorophylls a and b.



The cnidarian-dinoflagellate symbiosis is a nutritional mutualism. And thus the interaction between host and symbiont is mainly regulated by the exchange of inorganic and organic compounds. This graphic only shows the main flows, which include the uptake of dissolved inorganic carbon for symbiont photosynthesis and the exchange of organic compounds from the symbiont to the host as well as viceversa. Zooxanthellae are estimated to provide up to 90 percent of the coral's energy requirement in the form of photosynthetically fixed carbon.



## Coral bleaching – the collapse of symbiosis

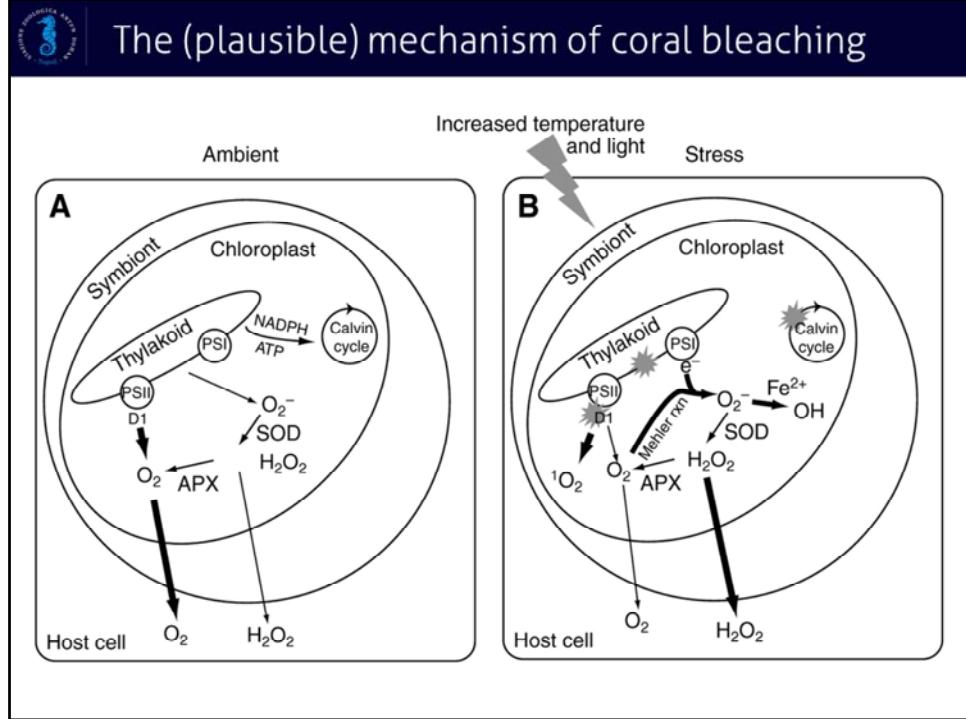


Negative environmental conditions, such as abnormally warm temperatures or high light can lead to the breakdown of the coral symbiosis. Under such stressful conditions, the coral host consumes or expels the zooxanthellae to ensure short-term survival. This leads to a lighter or completely white appearance, hence the term "bleaching".

Coral bleaching, if the stressful conditions persist, can lead to widespread coral mortality over vast areas.



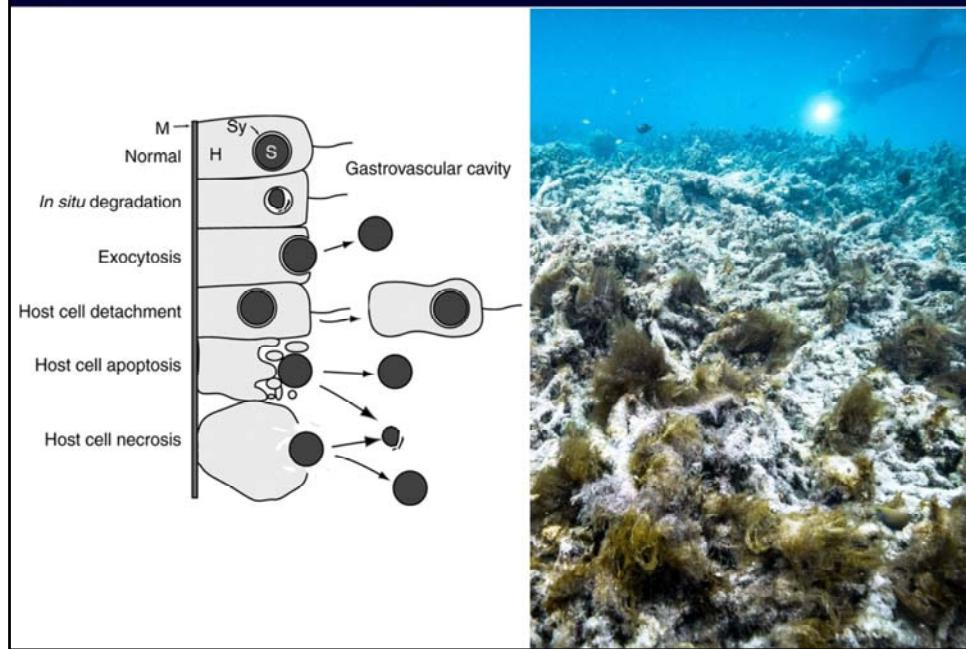
## The (plausible) mechanism of coral bleaching



The photosynthetic apparatus of the zooxanthellae operates normally under ambient conditions, producing large quantities of oxygen that diffuse into the host, where reactive oxygen species are converted back to oxygen by antioxidant enzymes. However, damage to the photosynthetic apparatus under stressful conditions acts to generate large amounts of ROS that overwhelms the antioxidant defense system in place in the symbiont, triggering a positive feedback loop.

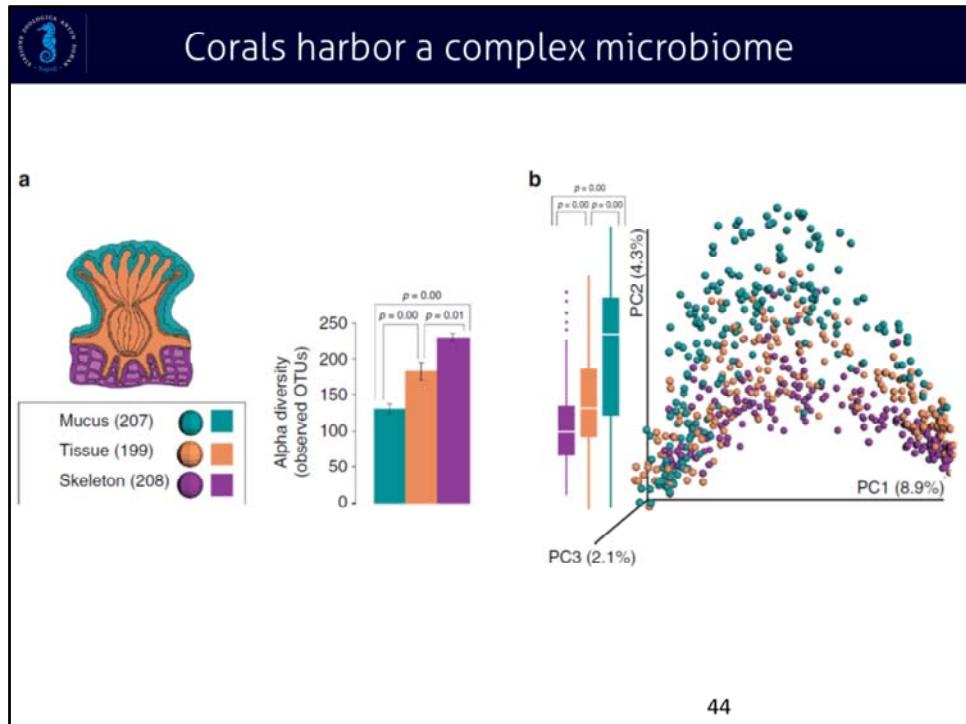


## The outcome(s) of coral bleaching



There are different types of cellular mechanisms of symbiont loss from the coral host tissues, either via degradation or expulsion of the symbiotic algae, or through complete host cell detachment, host cell apoptosis or even cell necrosis, the most serious outcome for the animal host.

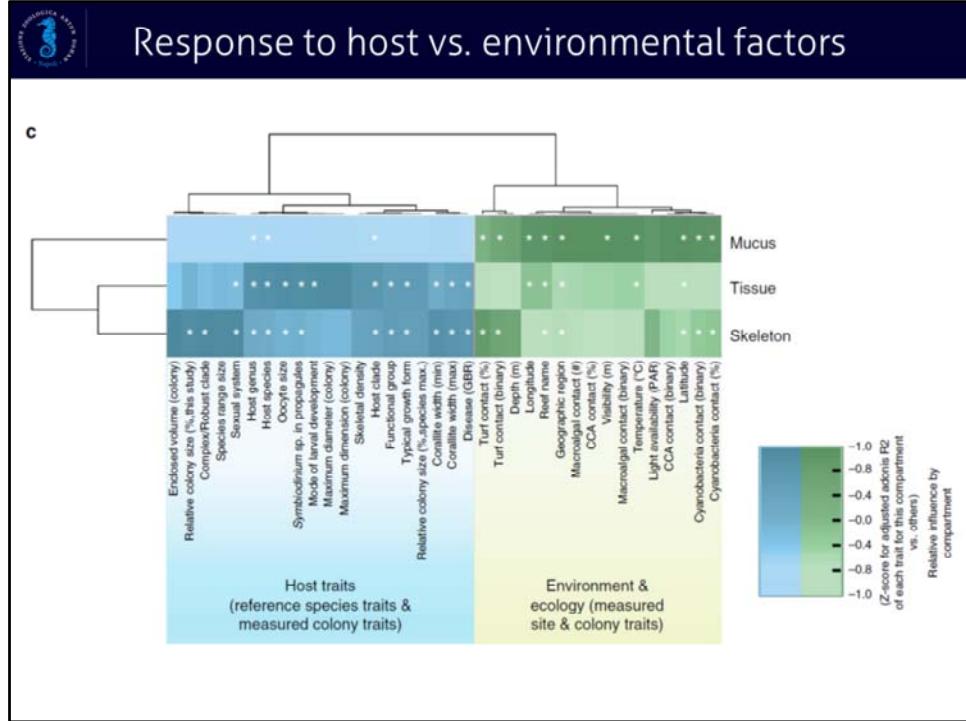
Depending on the level and type of stress, one or more of these processes can occur. If the stress is prolonged, bleaching will eventually lead to coral mortality with potentially scary outcomes as in this picture, where the entire reef is broken down to rubble.



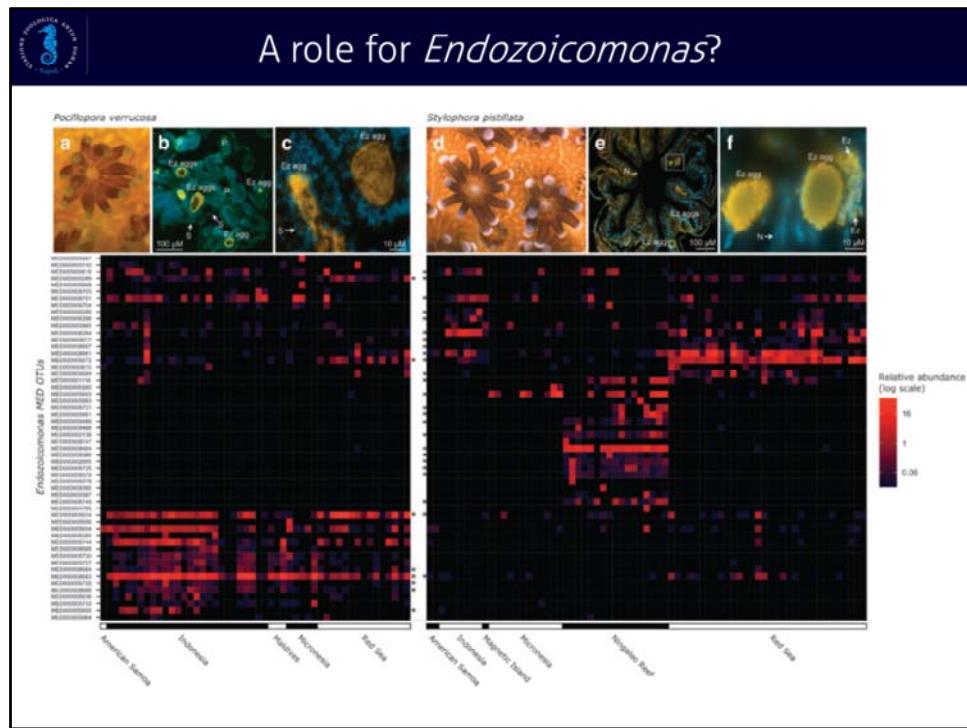
Corals harbor complex microbiomes, with a high phylogenetic diversity and many potential functions.

The anatomy of the animal host provides different microhabitats to the microbiome.

Indeed, here we see that microbiomes inhabiting the coral mucus, the tissue, and the skeleton differ both in richness (a) and in composition (b) based on analyses of 16S rRNA gene sequence data from phylogenetically diverse Australian corals.



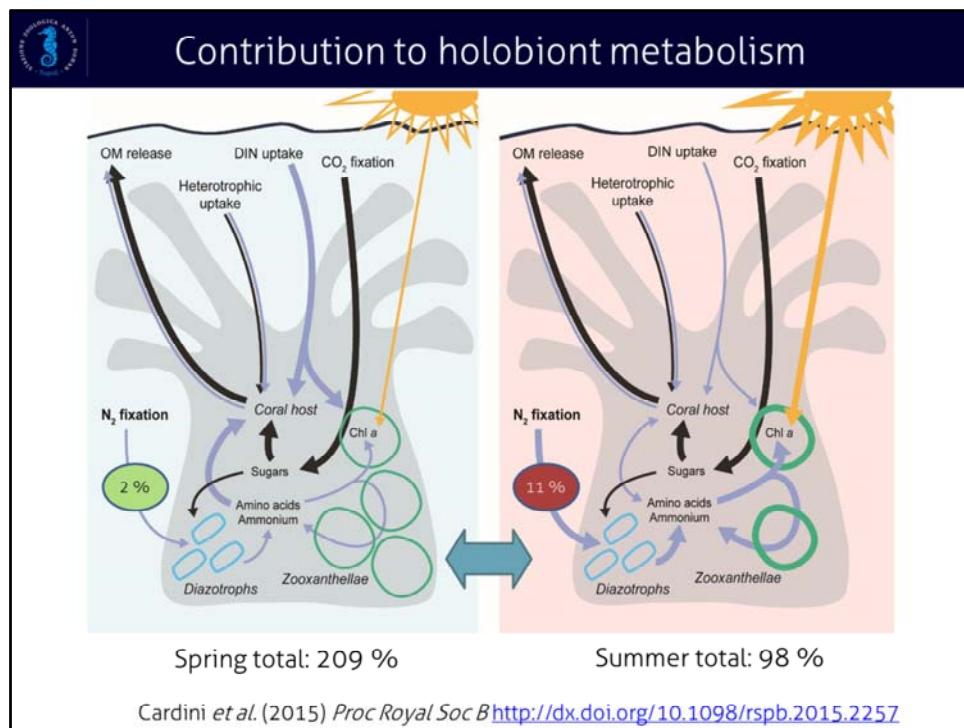
Coral mucus, tissue, and skeleton microbiomes differ also in response to host vs. environmental factors. While both host and environmental factors influenced all compartments, host factors tended to influence coral tissue and skeleton more strongly than mucus, whereas host environment more influenced mucus microbiomes.



Among this diverse community, *Endozoicomonas*, a gammaproteobacterium of the order Oceanospirillales, may play an important role as an endosymbiont of corals across species and space.

Here you see a figure with light microscopy and FISH imaging of *Endozoicomonas* within the tissues of *P. verrucosa* and *S. pistillata*, and a heatmap of *Endozoicomonas* OTU abundance and diversity in *P. verrucosa* and *S. pistillata* across different regions of the Indo-Pacific. *Endozoicomonas* bacteria appear yellow in the FISH images.

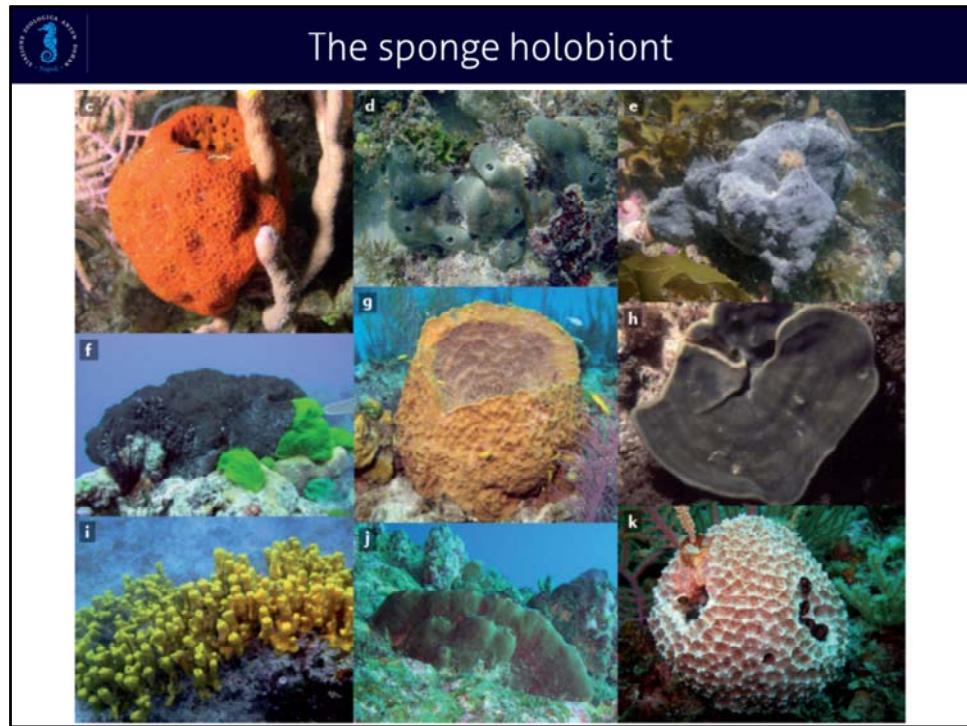
*P. verrucosa* harbors the same *Endozoicomonas*, whereas *S. pistillata* associates with geographically distinct genotypes. This specificity may be shaped by the different reproductive strategies of the hosts: spawning corals such as *P. verrucosa* acquire prokaryotes from the environment. In contrast, brooding corals such as *S. pistillata* release symbiont-packed planula larvae, which may explain a strong regional signature in their microbiome.



As already mentioned, the functions that the coral microbiome may play within their host are numerous, and studies looking at potential beneficial microbial processes in corals are difficult.

In previous work, I have approached the topic by measuring N<sub>2</sub> fixation in corals of the Red sea, and have demonstrated that this process which is peculiar of a limited group of prokaryotes, is carried out in corals and is of particular relevance during summer, when oligotrophic conditions cause the symbiosis to be N limited.

In such conditions, the contribution of N<sub>2</sub>-fixing prokaryotes can be important to fuel primary production by the zooxanthellae.



Sponges are a successful (> 8000 species) and evolutionarily ancient phylum, their members being globally distributed and abundant within the benthic communities of a wide range of habitats.

Their symbiosis with microorganisms has resulted in one of the most diverse and complex holobionts in the marine environment.

Here you can see the diversity in morphology among only a selected number of sponges that are used as model species.

Underwater photography of important model sponge species: *Mycale laxissima* (c), *Amphimedon queenslandica* (d), *Ancorina alata* (e), *Rhopaloeides odorabile* (f), *Xestospongia muta* (g), *Cymbastela concentrica* (h), *Aplysina aerophoba* (i), *Theonella swinhonis* (j) and *Ircinia felix* (k).



## Sponges: powerful filter feeders



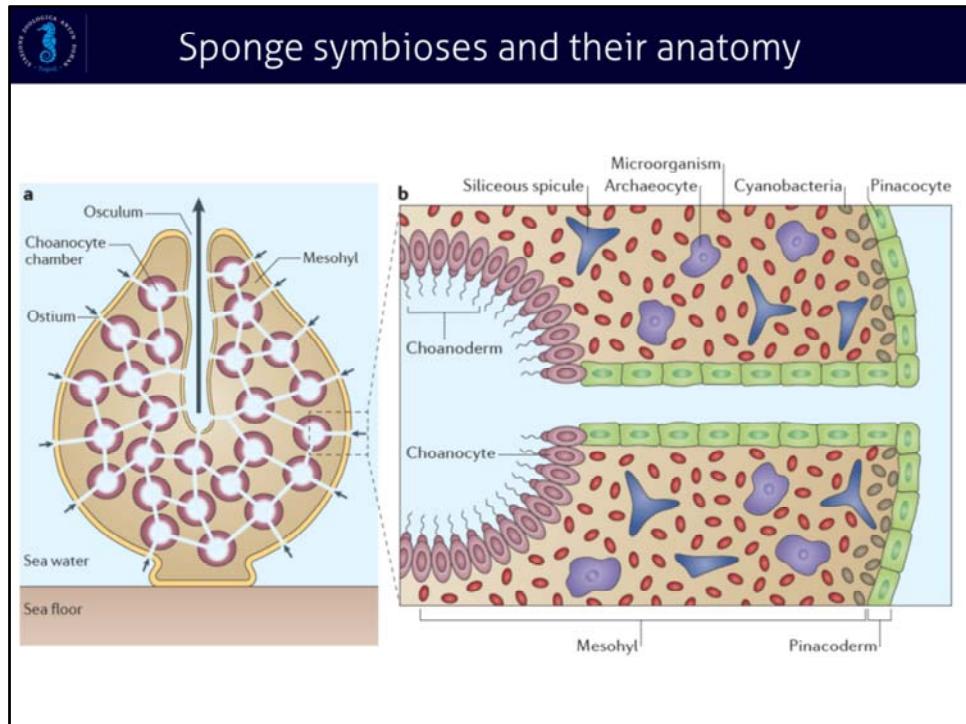
Sponges are filter feeders, capable of circulating thousands of liters of seawater through their osculum per day while feeding on organic particles and microorganisms from the water column. Thus, they are in constant contact with an enormous number of extremely diverse bacteria from the environment.

They feed on them, they select many of them for a resident symbiont population, which among other roles protect their host from pathogens.

Here you can see as fluorescent dye is steadily filtered through the sponges.



## Sponge symbioses and their anatomy

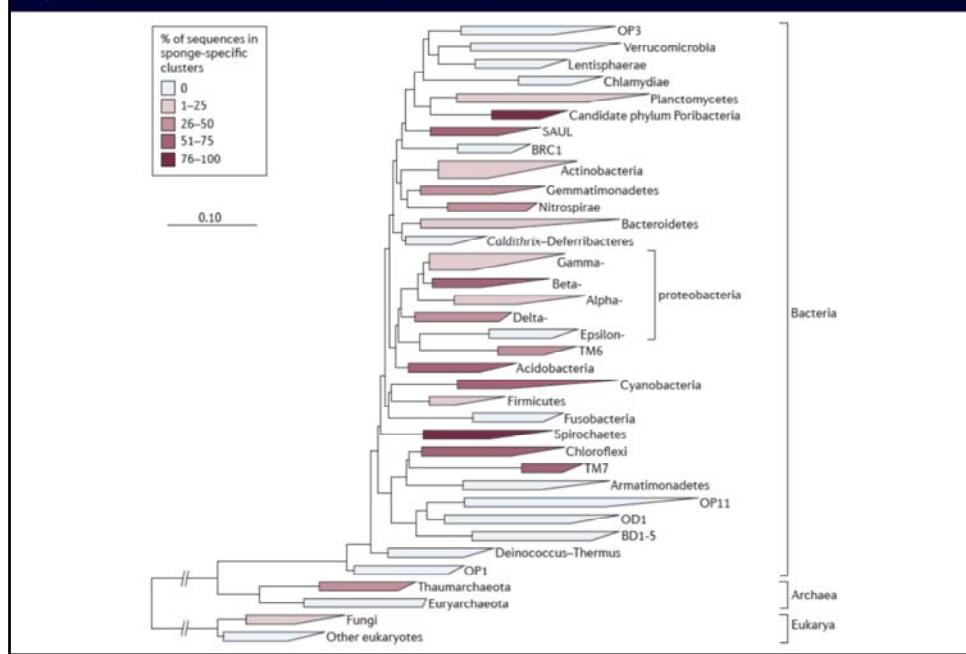


In this figure the anatomy of a sponge is represented, and you can see the inhalant openings (ostia) through which water enters and is filtered (up to 96% of microbial cells are removed!!) to then be discharged via the exhalant opening (osculum).

Within the sponge, microorganisms accumulate in an inner tissue layer called mesohyl.

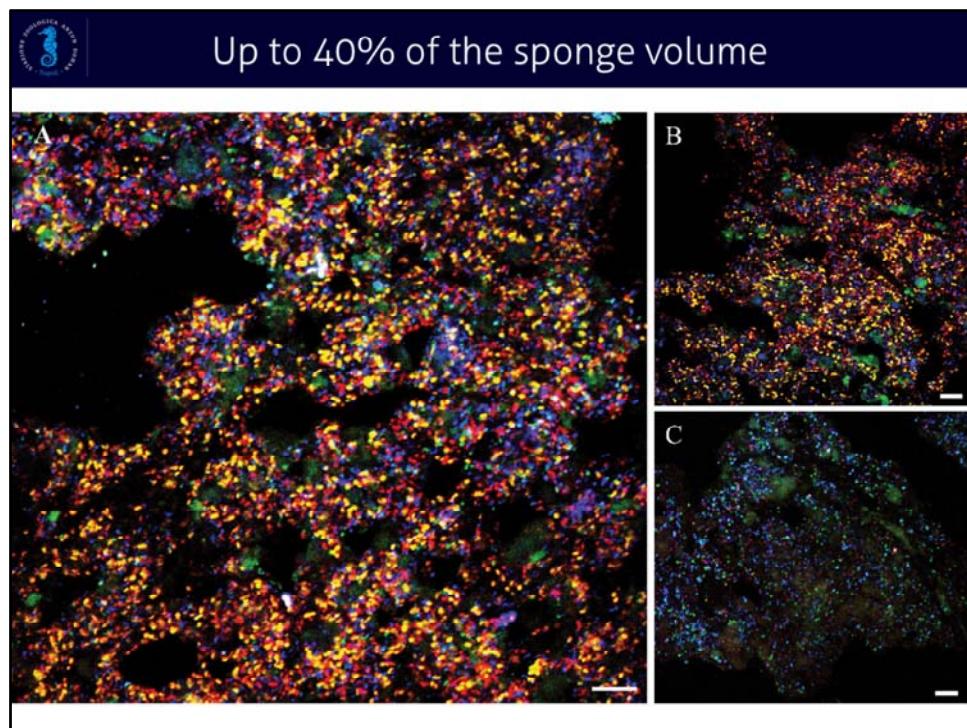


## A striking phylogenetic diversity



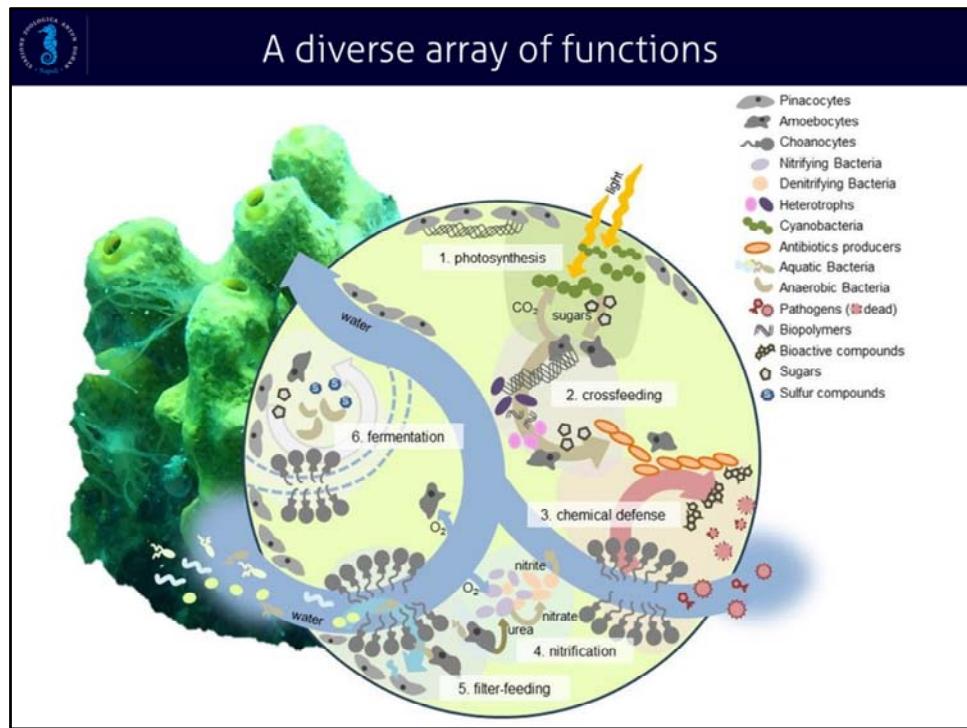
Rather than ‘one host, one symbiont’ associations, sponges represent ‘many hosts, many symbionts’ associations, probably as a result of the ample exchange with sea water that occurs during filter feeding, and also the lack of microbial compartmentation to specific cells or organs.

Marine sponges contain a striking phylogenetic diversity of microorganisms, as you can see from 16S rRNA gene-based phylogenetic analysis of the sponge microbiota showing the diversity and specificity of marine sponge-associated microorganisms.



Microbes can comprise as much as 40% of sponge tissue volume, with densities in excess of  $10^9$  microbial cells per ml of sponge tissue, several orders of magnitude higher than those typical for seawater!!!

In this figure, a FISH using double labeling of oligonucleotide probes (called DOPE-FISH) is used to highlight the diversity and abundance of sponge symbionts. In the big frame, yellow highlights Poribacteria, pink Nitrospira, cyan Chloroflexi, green Deltaproteobacteria, red Gammaproteobacteria, blue Archaea.



A diverse microbial community often means a wide array of possible functions. Here represented are the main ones, with also some key microbial groups indicated.

For example, photosynthesis by cyanobacteria can generate important carbon sources for the sponge or other microbes.

Nitrification is another important biogeochemical transformation that often is found taking place in sponges.

The associated microbial community also produces and secretes a variety of bioactive compounds for chemical defense against pathogens.

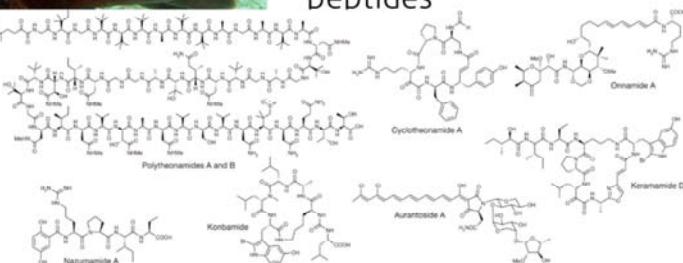


## Marine drugs from sponges



Each individual of *Theonella swinhonis* contains hundreds of bacterial symbiont phylotypes

>40 distinct bioactive polyketides and modified peptides



Indeed sponges are a precious source of new molecules in the search of new drugs.

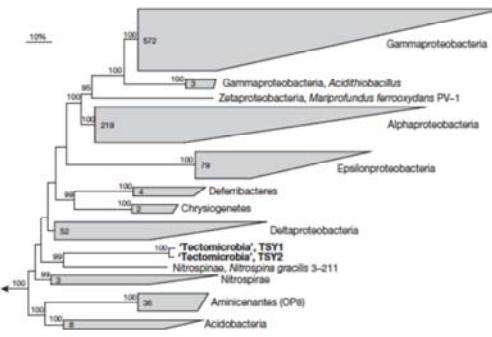
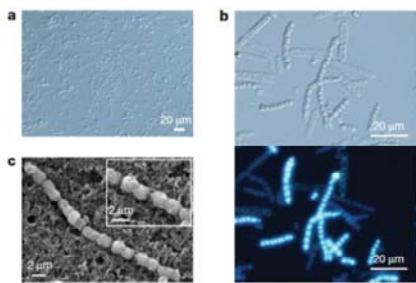
For example, each individual of *Theonella swinhonis* contains hundreds of bacterial symbiont phylotypes, which occupy up to 40% of its body volume. The species produces more than 40 distinct bioactive secondary metabolites of biomedical interest such as polyketides and modified peptides.



## *Candidatus*Entotheonella factor

Genome size: >9 Mb

Encodes most of the known polyketides and peptides from this sponge species



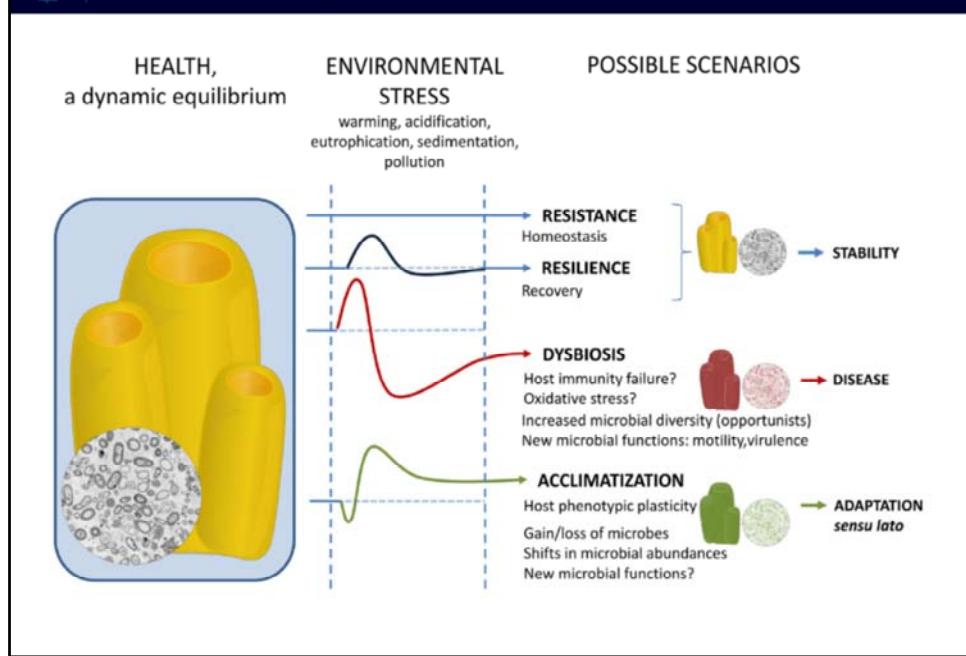
A novel candidate phylum 'Tectomicrobia'  
-*tegere*, to hide, to protect-

A recent study using single-cell genomics and metagenomics found that almost all bioactive polyketides and peptides known from this animal were attributed to a single phylotype, for which the name *Entotheonella factor* was proposed (latin, factor; the producer)

The species is affiliated to a novel candidate phylum named 'Tectomicrobia' (latin *tegere*, to hide, to protect) because of its capability to produce bioactive compounds that are likely used as chemical defence.

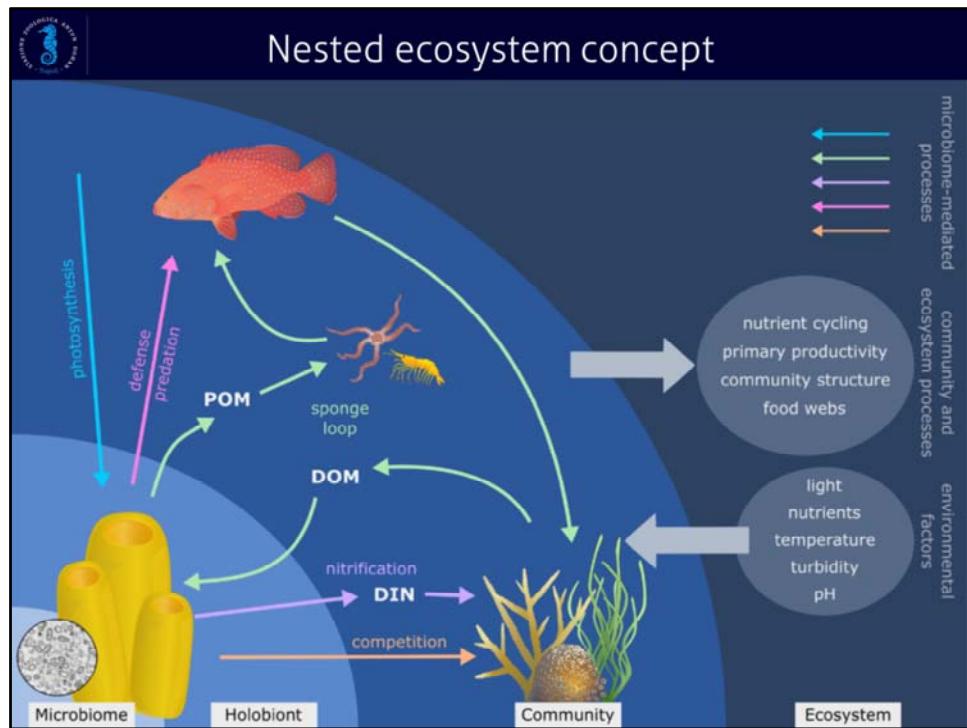


## Microbial symbioses and holobiont health



Sponges are a perfect example of a complex holobiont, where health is regarded as a dynamic equilibrium balanced by the host, the microbiome, as well as the interaction between them.

Understanding the underlying principles of health and holobiont dynamics would help predict the responses upon perturbation and whether the final outcome will allow stability, yield disease, or turn into an opportunity for adaptation.



By associating with an eukaryotic host, microbial symbionts boost their physiologies and escape competition, potentially contributing significantly to ecosystem processes. This is what

In marine systems, holobionts are further integrated into larger and more complex communities and ecosystems, a concept referred to as “nested ecosystems.”

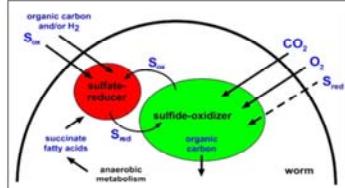
Key functions mediated in one way or another by the microbiome (colored arrows) influence holobiont functioning and, through cascading effects, subsequently influence community structure and ecosystem functioning.

Environmental factors act at multiple scales to alter microbiome, holobiont, community, and the ecosystem.

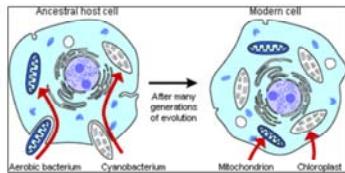
Thus, factors that alter microbiome functioning can lead to changes at the holobiont, community, or even ecosystem level and vice versa, illustrating the necessity of considering multiple scales when evaluating functioning in nested ecosystems.



## Take home: microbial symbioses as...



A source of novel capabilities



A determinant in the evolution of eukaryotes



A force for the ecological success of plants and animals, and of entire ecosystems

To conclude, our take home messages for today are that microbial symbioses are not only ubiquitous in the natural world, but act upon different organizational scales providing

a source for novel capabilities (think of augmented metabolisms in chemosynthetic symbioses)

a determinant in the evolution of eukaryotes (think of the endosymbiotic theory)

but also a force for the ecological success of plants and animals, and eventually of entire ecosystems (think of corals).



## References and further resources

### Fantastic books:

Ed Yong. *I Contain Multitudes: The Microbes Within Us and a Grander View of Life*, 355 pp (Ecco Pr, 2016)  
<https://www.amazon.it/Contain-Multitudes-Microbes-Within-Grander/dp/0062368591>

Angela E. Douglas. *The Symbiotic Habit*, 214 pp (Princeton University Press, 2010)  
<https://press.princeton.edu/books/paperback/9780691113425/the-symbiotic-habit>

### Relevant references:

Beinart (2019) <https://doi.org/10.1128/mSystems.00127-19>  
Bright M, Bulgheresi S (2010) <https://doi.org/10.1038/nrmicro2262>  
Douglas AE, Werren JH (2016) <https://doi.org/10.1128/mBio.02099-15>  
Estrela S, Kerr B, Morris JJ (2016) <https://doi.org/10.1016/j.mib.2016.04.007>  
McCutcheon JP, Boyd BM, Dale C (2019) <https://doi.org/10.1016/j.cub.2019.03.032>  
McFall-Ngai M, et al. (2013) <https://doi.org/10.1073/pnas.1218525110>  
Moran NA, Sloan DB (2015) <https://doi.org/10.1371/journal.pbio.1002311>  
Sachs JL, Quides KW, Wendlandt CE (2018) <https://doi.org/10.1111/nph.15222>  
Shapira M (2016) <https://doi.org/10.1016/j.tree.2016.1003.1006>  
Zachar I, Boza G (2020) <https://doi.org/10.1007/s00018-020-03462-6>  
Wein T, et al. (2019) <https://doi.org/10.1016/j.tim.2019.05.010>

### Own work:

Cardini U, et al. (2015) <https://doi.org/10.1098/rspb.2015.2257>  
Cardini U, et al. (2019) <https://doi.org/10.1038/s41396-019-0486-9>  
Petersen JM, et al. (2017) <https://doi.org/10.1038/nmicrobiol.2016.195>

59

Here you can find some key resources if you want to delve into the world of microbial symbioses, and a couple of references from my own work.



## References and further resources

### Corals:

- Davy SK, Allemand D, Weis VM (2012) <https://doi.org/10.1128/MMBR.05014-11>  
Neave MJ, *et al.* (2017) <https://doi.org/10.1038/ismej.2016.95>  
Pollock FJ, *et al.* (2018) <https://doi.org/10.1038/s41467-018-07275-x>  
Weis VM (2008) <https://doi.org/10.1242/jeb.009597>

### Sponges:

- Hentschel U, Piel J, Degnan SM, Taylor MW (2012) <https://doi.org/10.1038/nrmicro2839>  
Webster NS, Thomas T (2016) <https://doi.org/10.1128/mBio.00135-16>  
Wilson MC, *et al.* (2014) <https://doi.org/10.1038/nature12959>

### Squid:

- McFall-Ngai M (2008) <https://doi.org/10.1016/j.cub.2008.08.059>  
Miyashiro T, Ruby EG (2012) <https://doi.org/10.1111/j.1365-2958.2012.08065.x>  
Nyholm SV, McFall-Ngai M (2004) <https://doi.org/10.1038/nrmicro957>

### Chemosynthetic symbioses:

- Dubilier N, Bergin C, Lott C (2008) <https://doi.org/10.1038/nrmicro1992>  
Sogin EM, Leisch N, Dubilier N (2020) <https://doi.org/10.1016/j.cub.2020.07.050>  
Stewart FJ, Newton ILG, Cavanaugh CM (2005) <https://doi.org/10.1016/j.tim.2005.07.007>

Dr. Ulisse Cardini – [ulisse.cardini@szn.it](mailto:ulisse.cardini@szn.it)  
Head of the Marine Symbiomes Research Group – [www.marinesymbiomes.eu](http://www.marinesymbiomes.eu)

And here some organism-specific references, and again my contact information.

Thank you for following this lecture, I hope you enjoyed it!