Developmental Symbiosis

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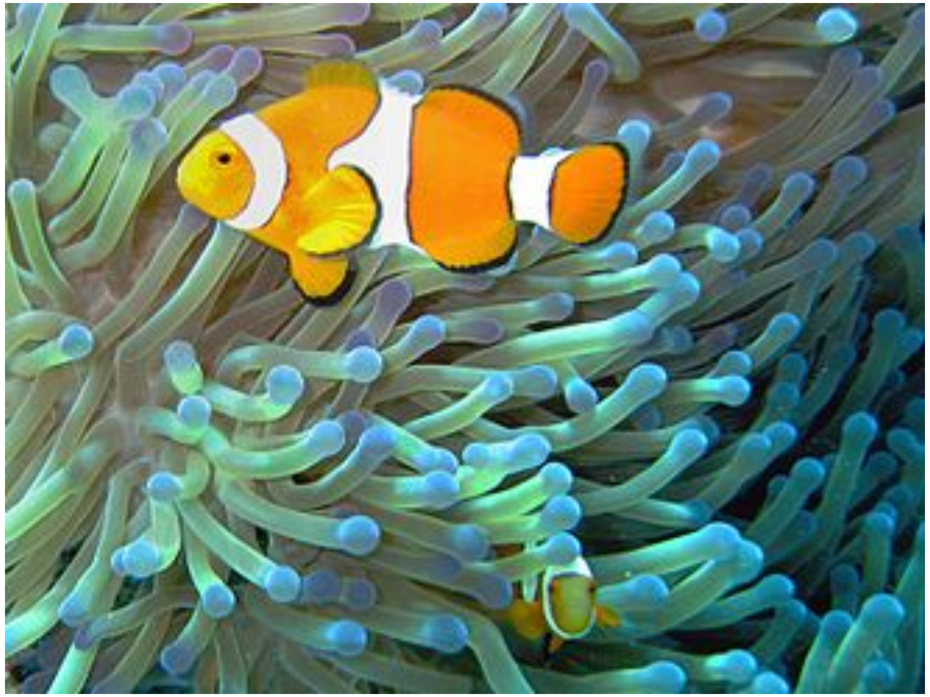
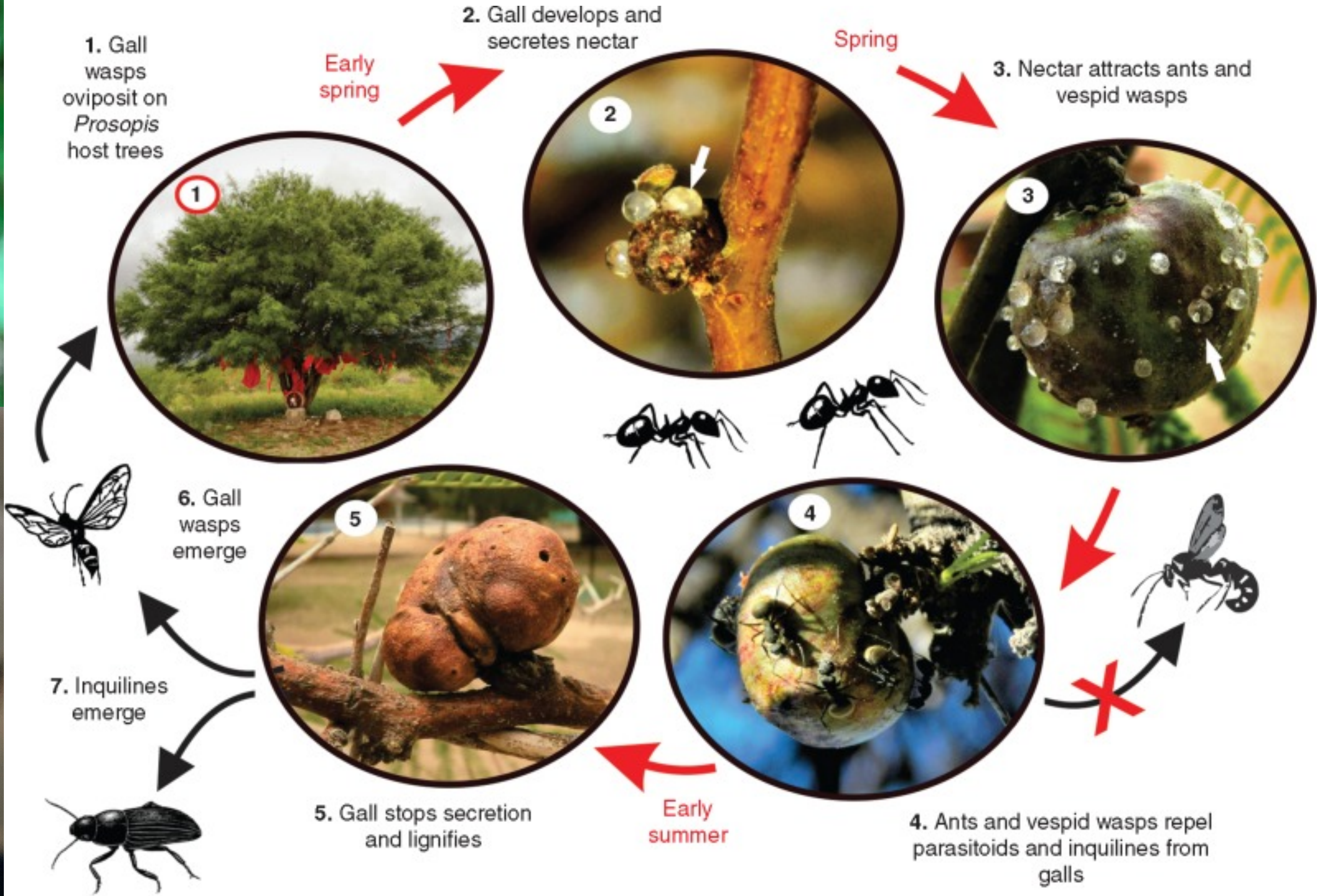
EvoDevo 2021

Background

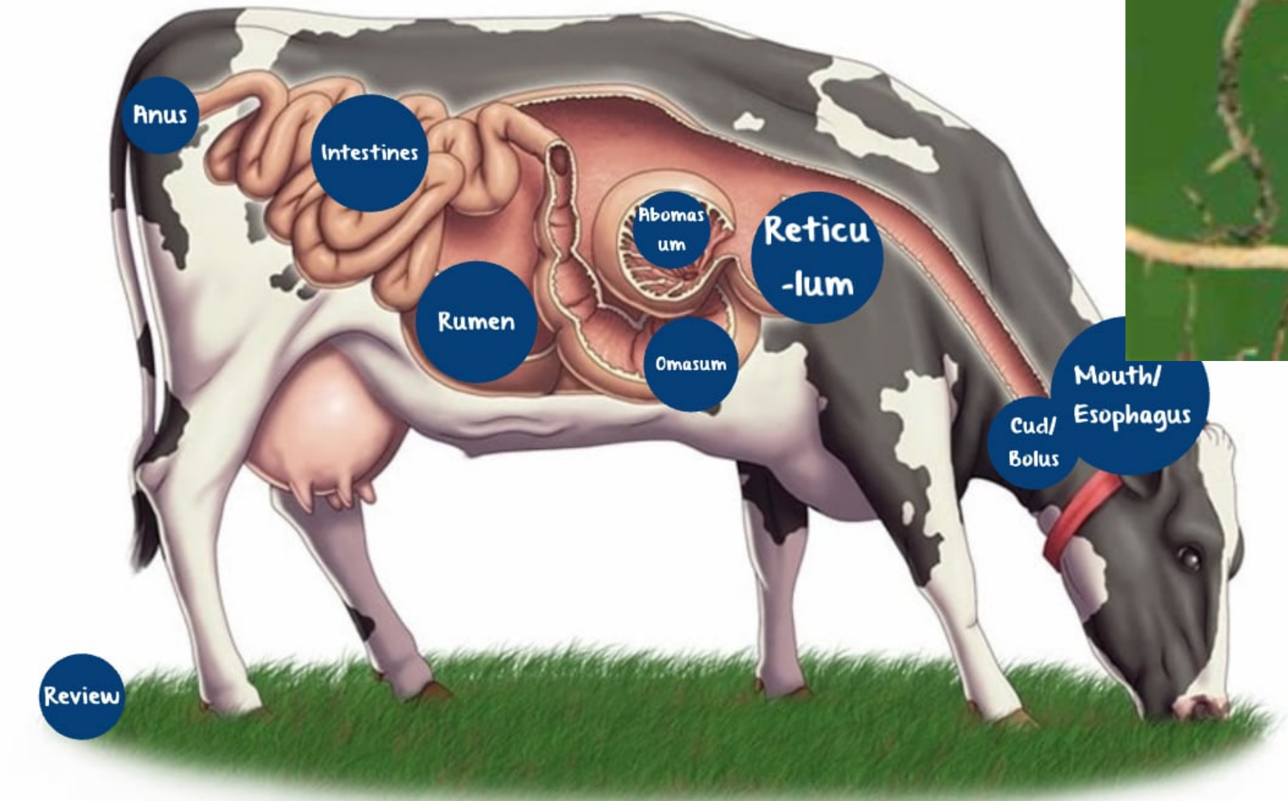
- A symbiotic relationship is a close and long-term biological relationship between two different organisms
  - Can be mutualistic, commensalistic, or parasitic




1. Gall wasps oviposit on *Prosopis* host trees
Early spring
2. Gall develops and secretes nectar
Spring
3. Nectar attracts ants and vespid wasps
4. Ants and vespid wasps repel parasitoids and inquilines from galls
Early summer
5. Gall stops secretion and lignifies
6. Gall wasps emerge
7. Inquilines emerge
1
2
3
4
5

Microbiome

- Eukaryotes have always been in contact with bacteria
- Through co-evolution since the formation of multicellular species, eukaryotes form a complex of host cells and many associated microorganisms
  - Bacteria, algae, archaea, fungi, protists, and viruses
- Contribute to a range of host function like nutrition, behavior, and development
  - Ruminant animals cannot produce enzymes to digest cellulose or lignin so the plant matter they eat must be fermented by microbes
  - Plants, especially legumes, utilize nitrogen-fixing bacteria

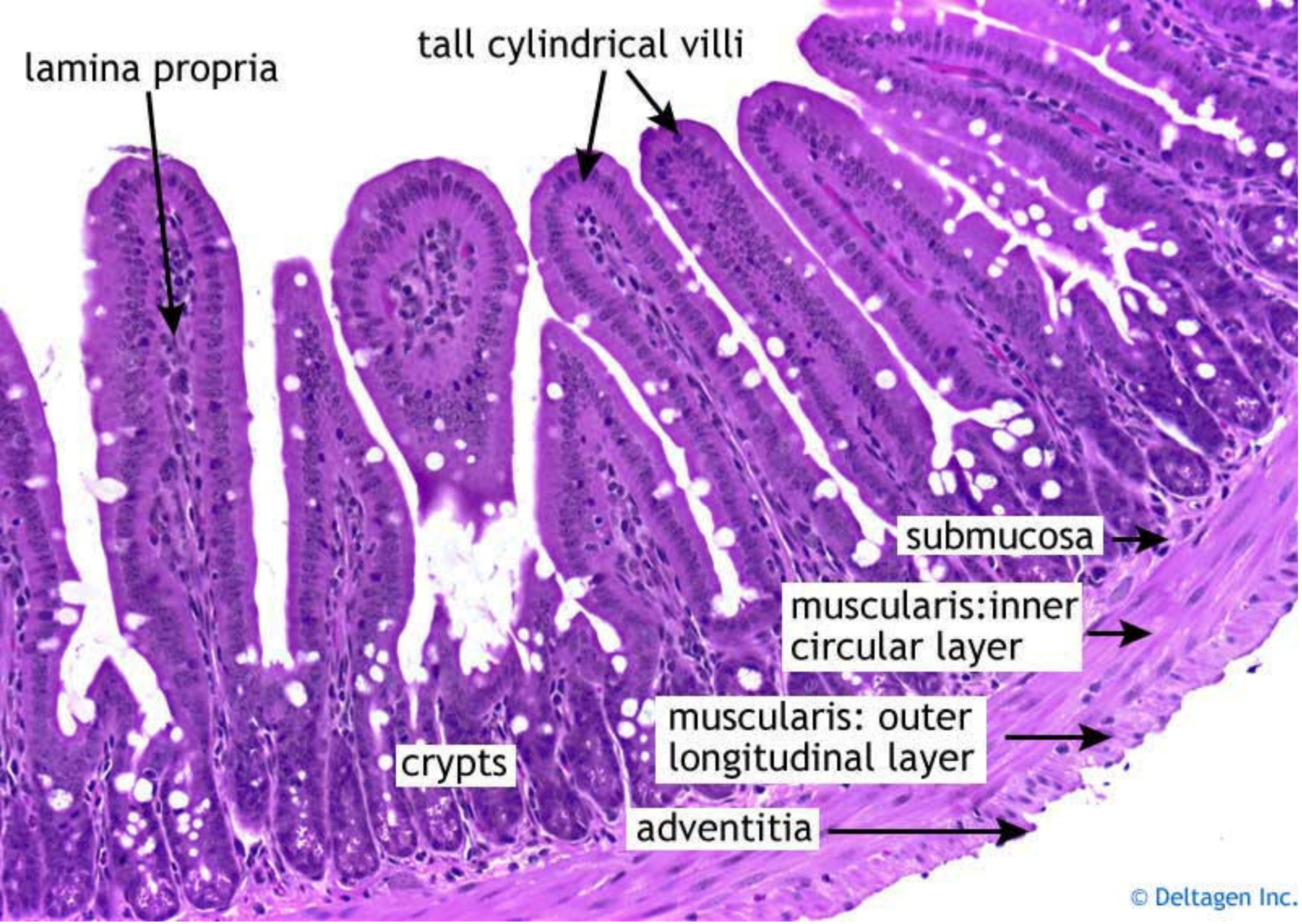

Anus
Intestines
Abomasum
Reticu-lum
Rumen
Omasum
Mouth/ Esophagus
Cud/ Bolus
Review

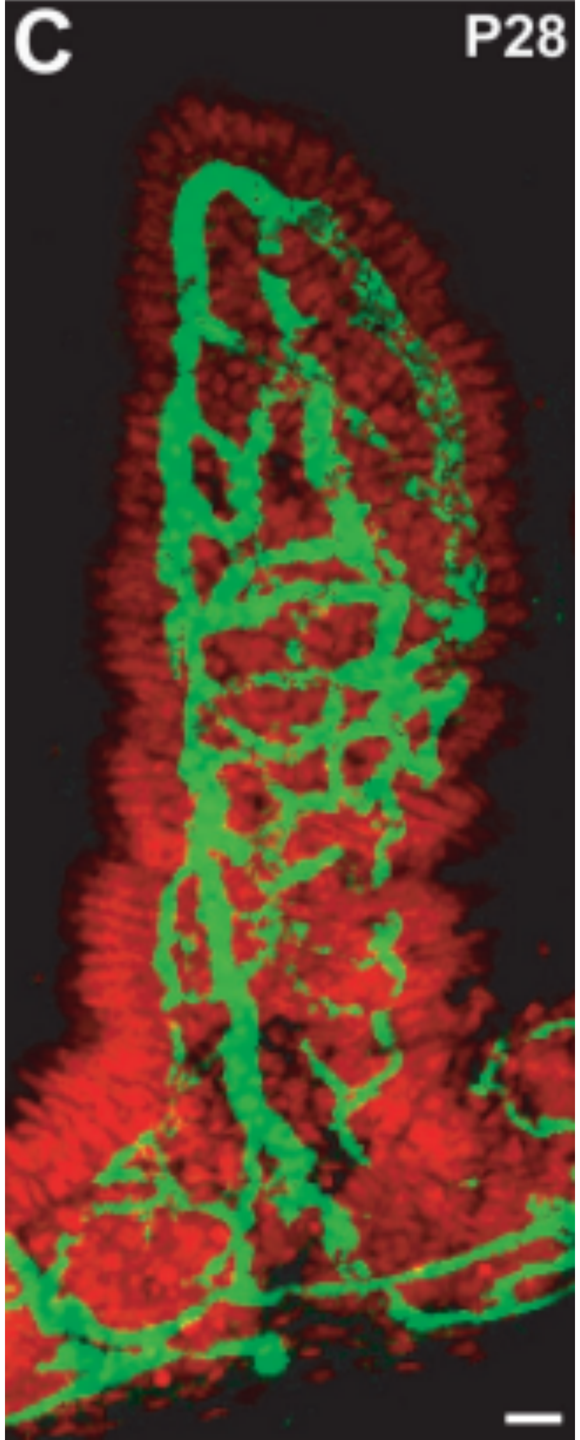
Holobiont / Hologenome

- This interdependence between multicellular host and symbiotic bacteria and other microbes can be though of as one “superorganism” or “evolutionary unit” known as the holobiont
    - Co-evolved species assemblages
- The collection of genomes from organisms that make up the holobiont is the hologeneome
- Symbiont cell count and genome size, both in terms of number of distinct genes and copies of each gene, are often much larger than host cell or genome size

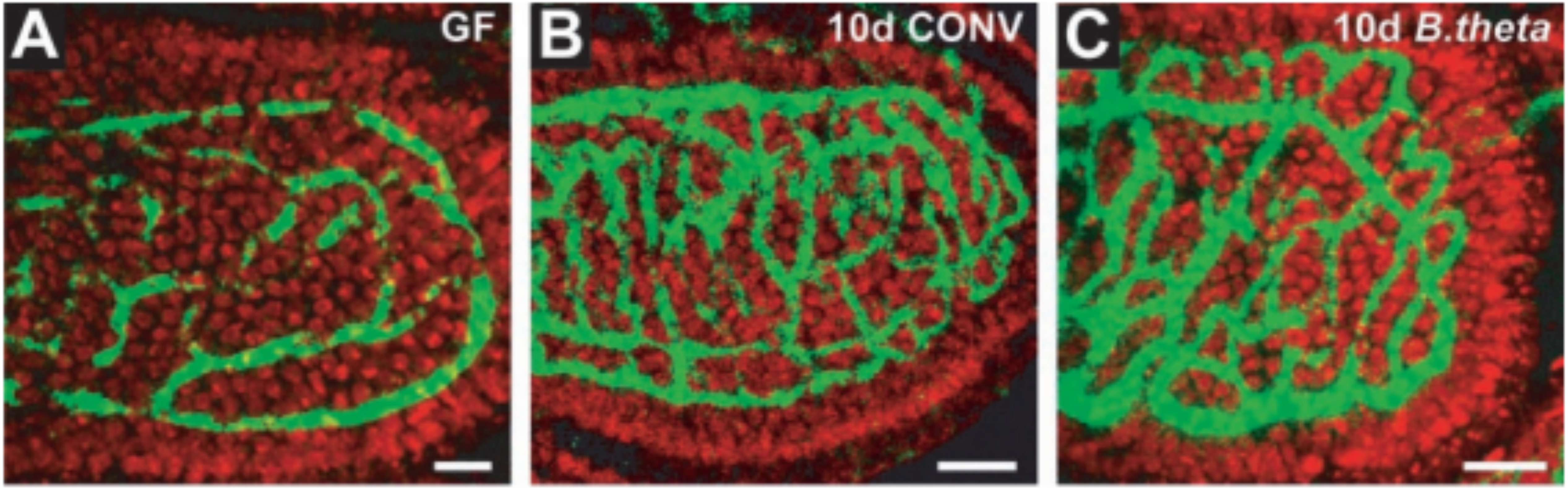
Background/Functions

- Human gut microbiome has been a huge area of recent research
  - It has been shown to play a large role in metabolism, immune defense, behavior, and development
- Germ-free zebrafish and mice develop smaller, immature guts
- They are unable to fully complete differentiation
- Going to look at a study of how microbiome affect development of vascular systems in the gut





- Microbial symbionts in the gut play a key role in the development of the mesenchymal microvascular network in the villi of the gut

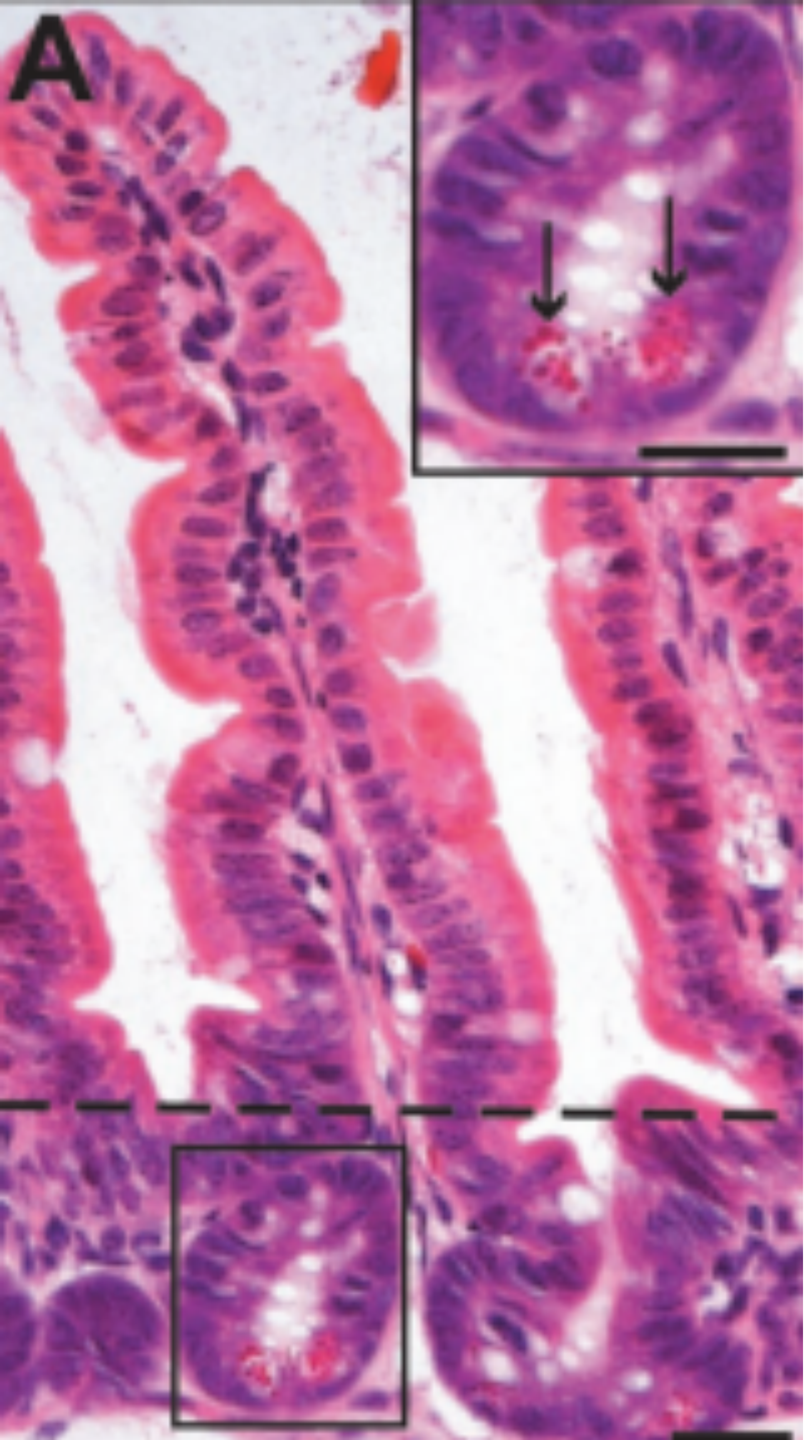


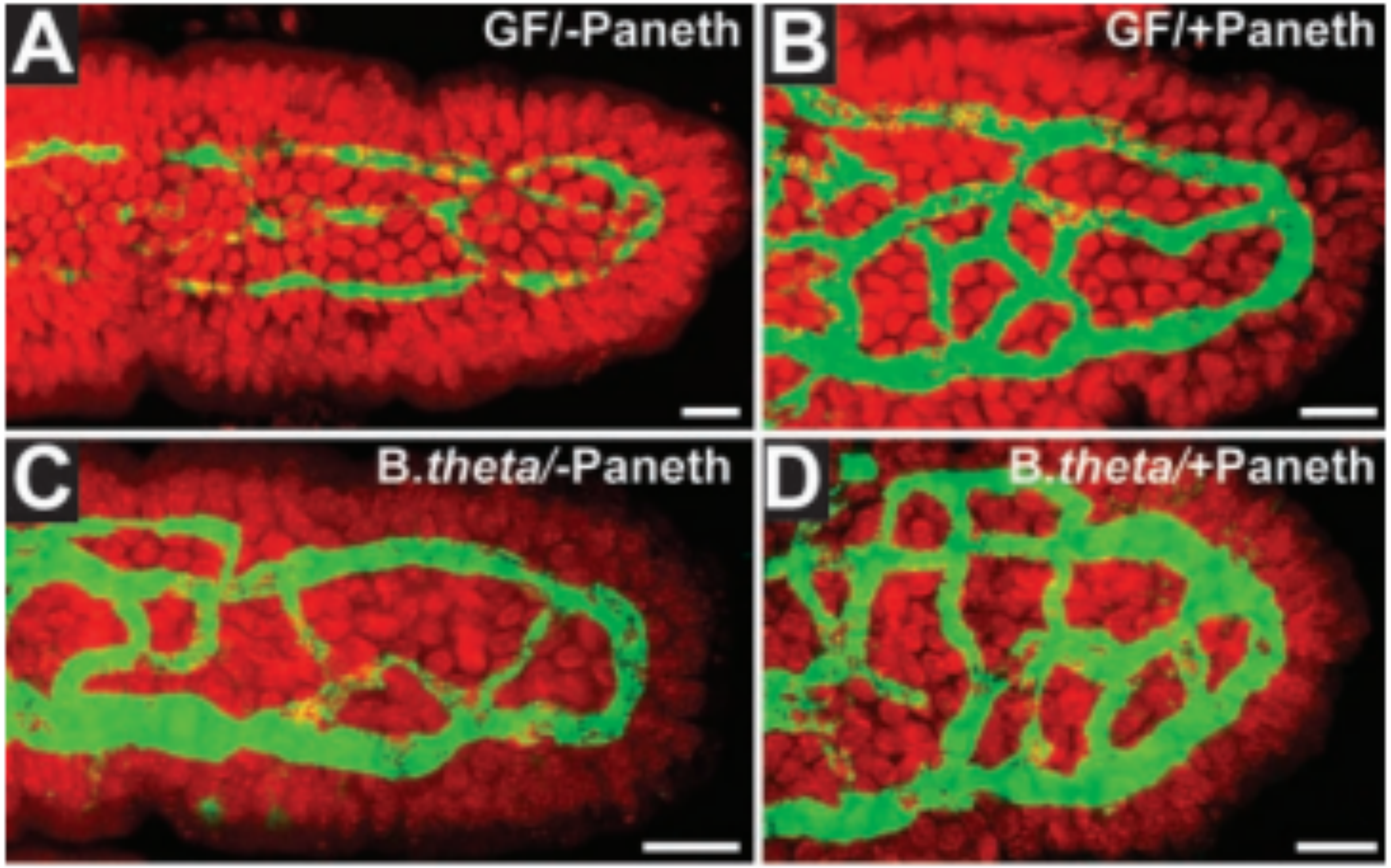
Germ-Free

Conventionally Reared

Inoculated

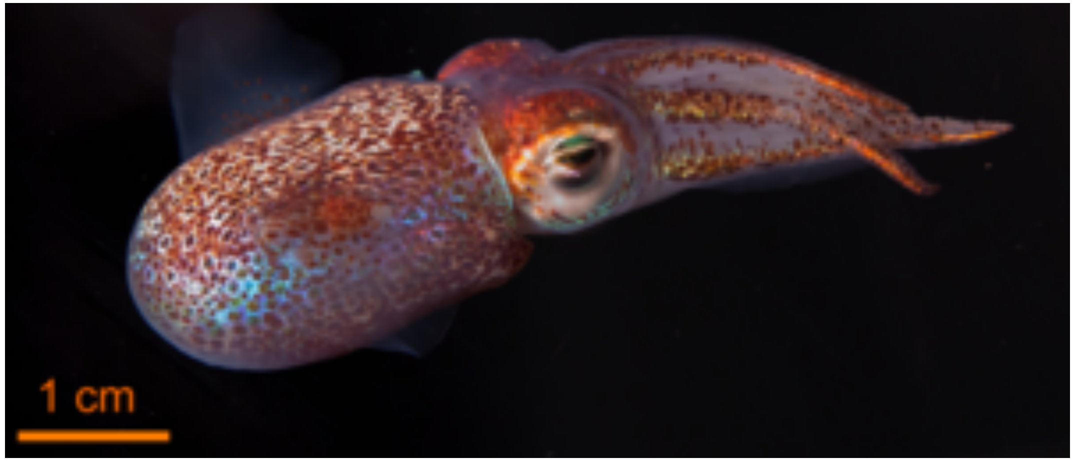
- Germ-free mice have less microvasculature in the villi (stained green) compared to conventionally raised and inoculated mice

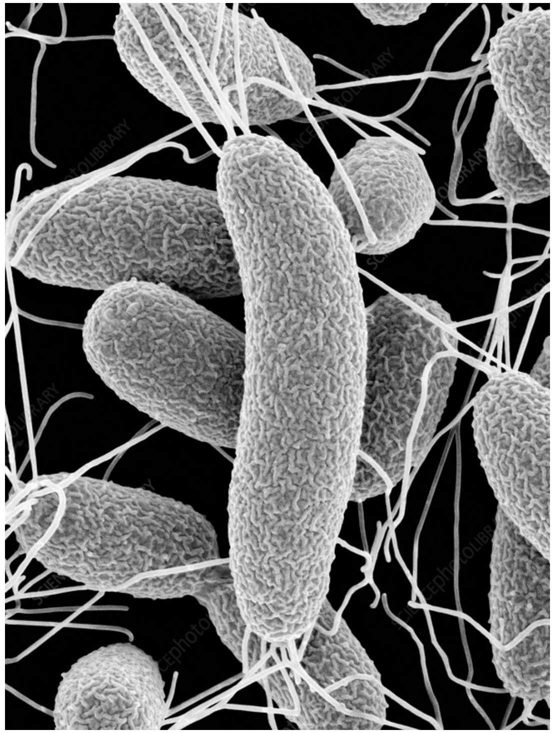


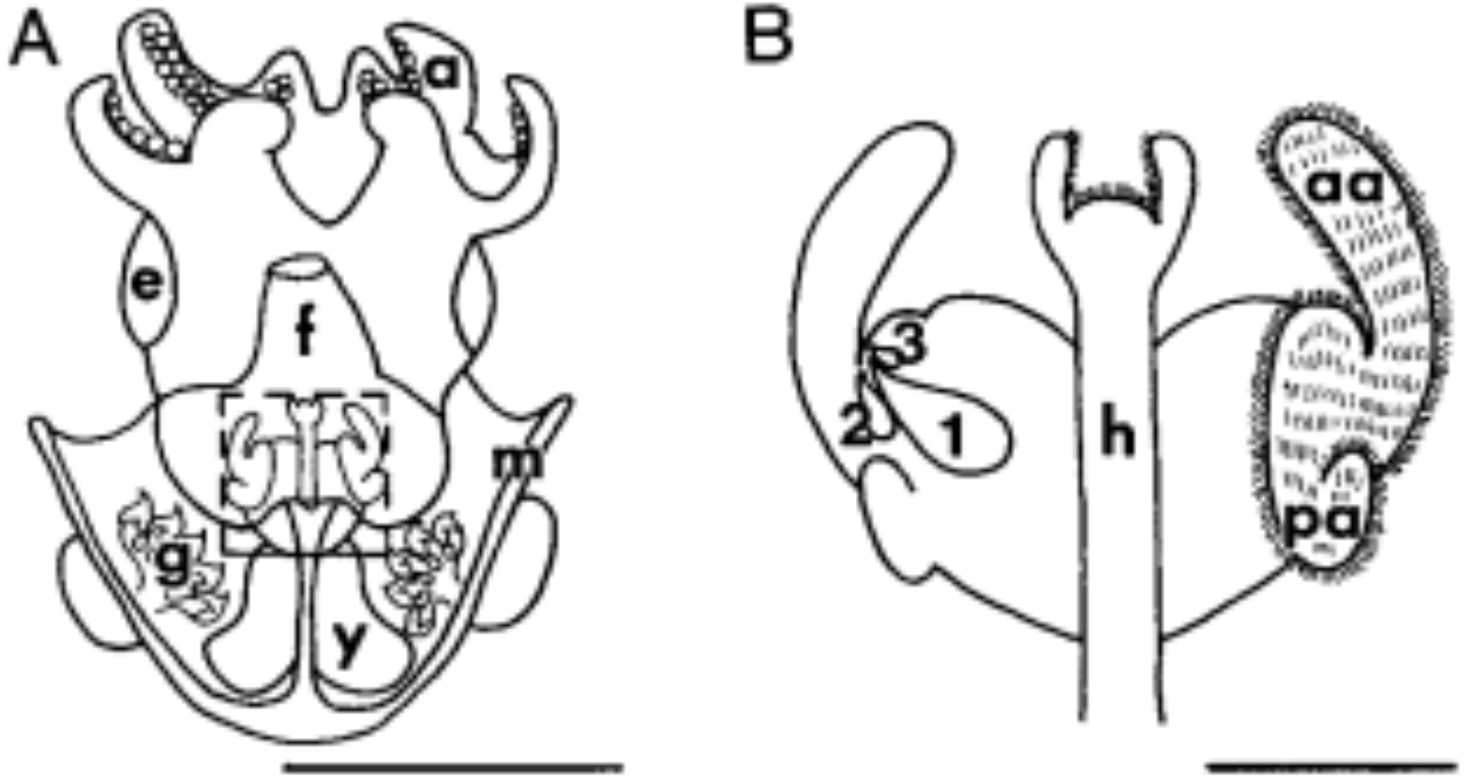


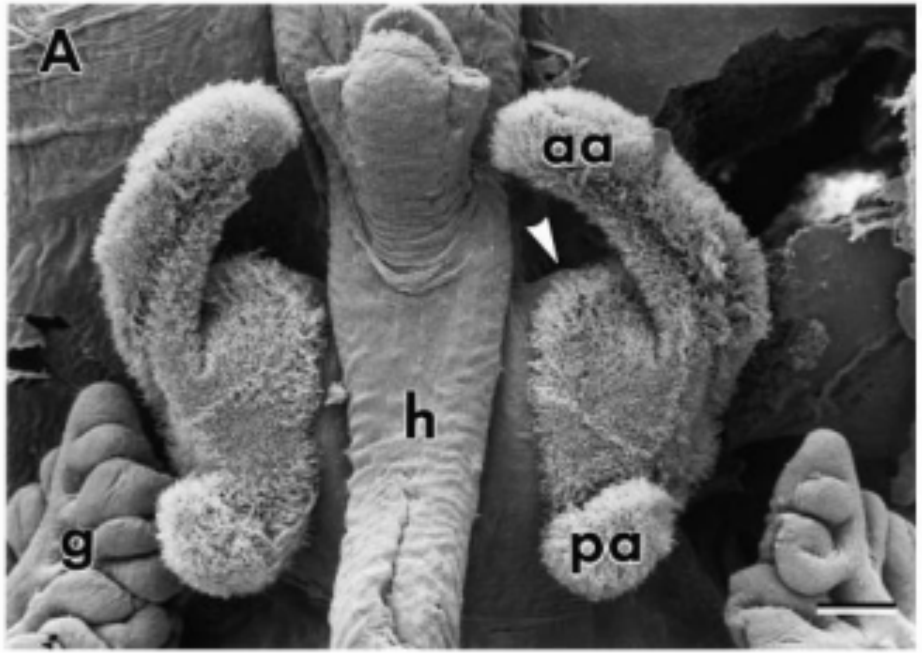
- Paneth cells also play a key role
  - Critical components of the innate immune system
  - Release antimicrobial peptides and proteins into the gut
  - Produce factors that play a key role in development of gut microvasculature

- The Hawaiian bobtail squid *Euprymna scolopes* has a symbiotic organ in which it houses *Vibrio fischeri* bacteria to bioluminesce

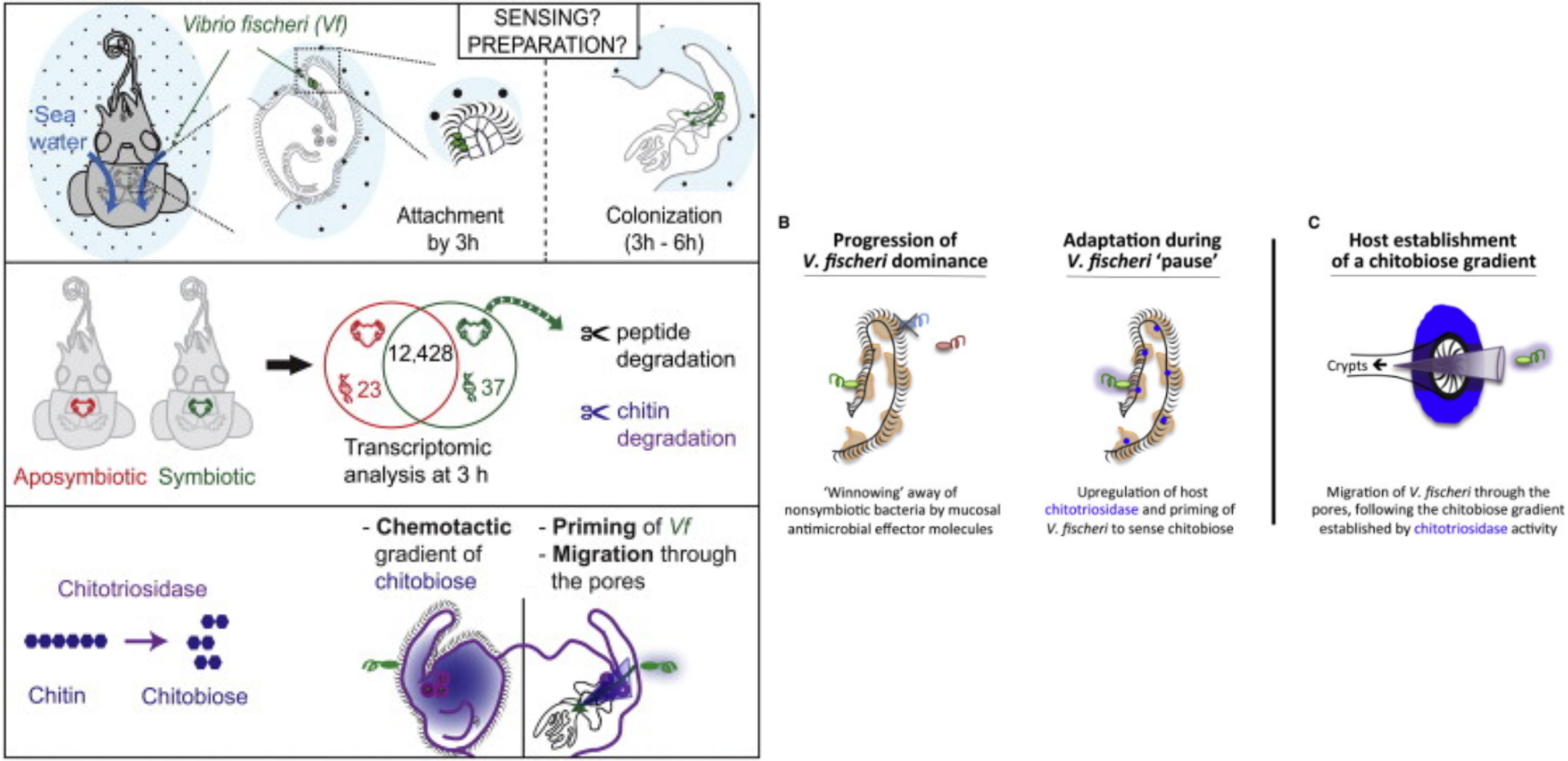




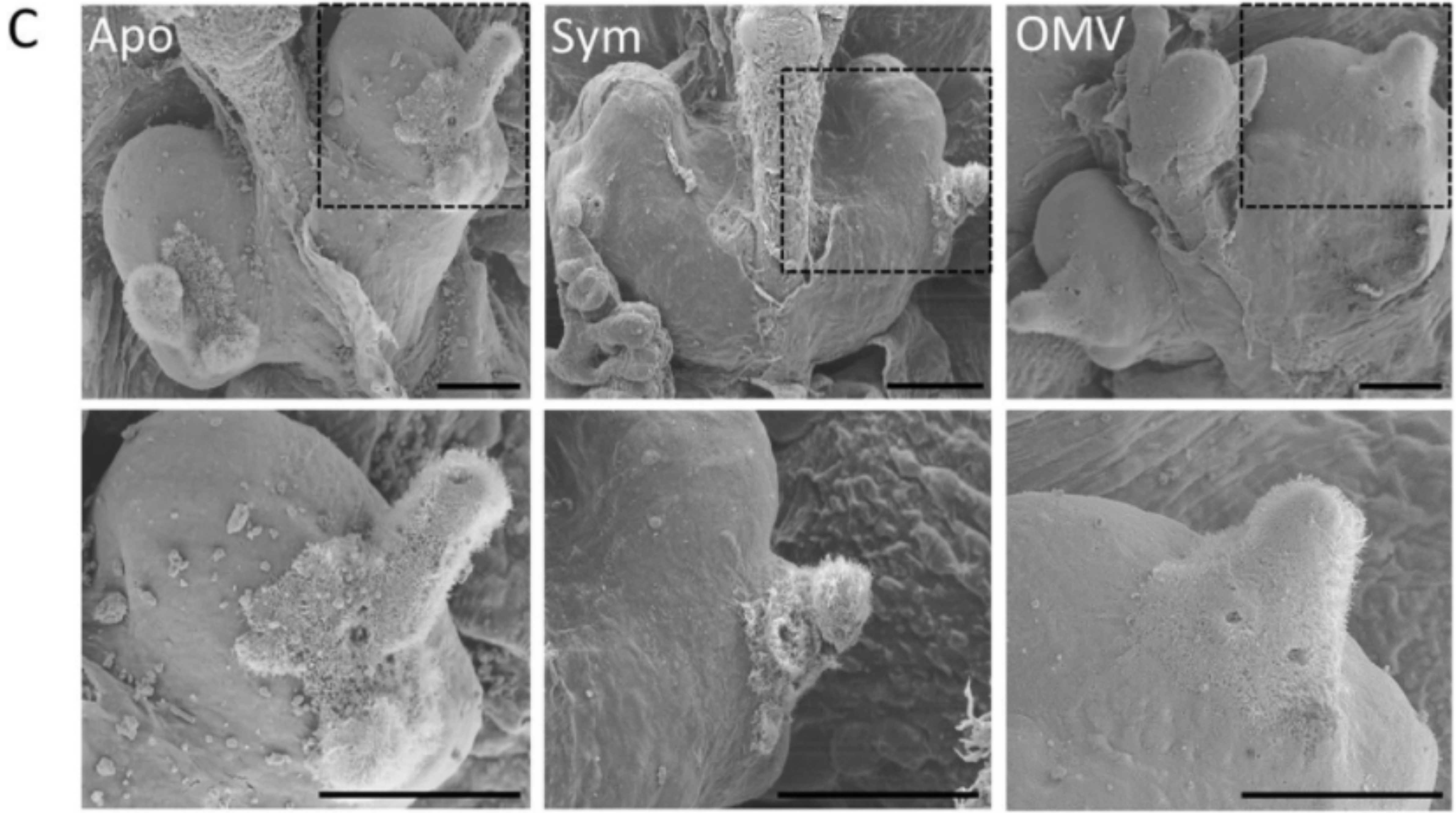


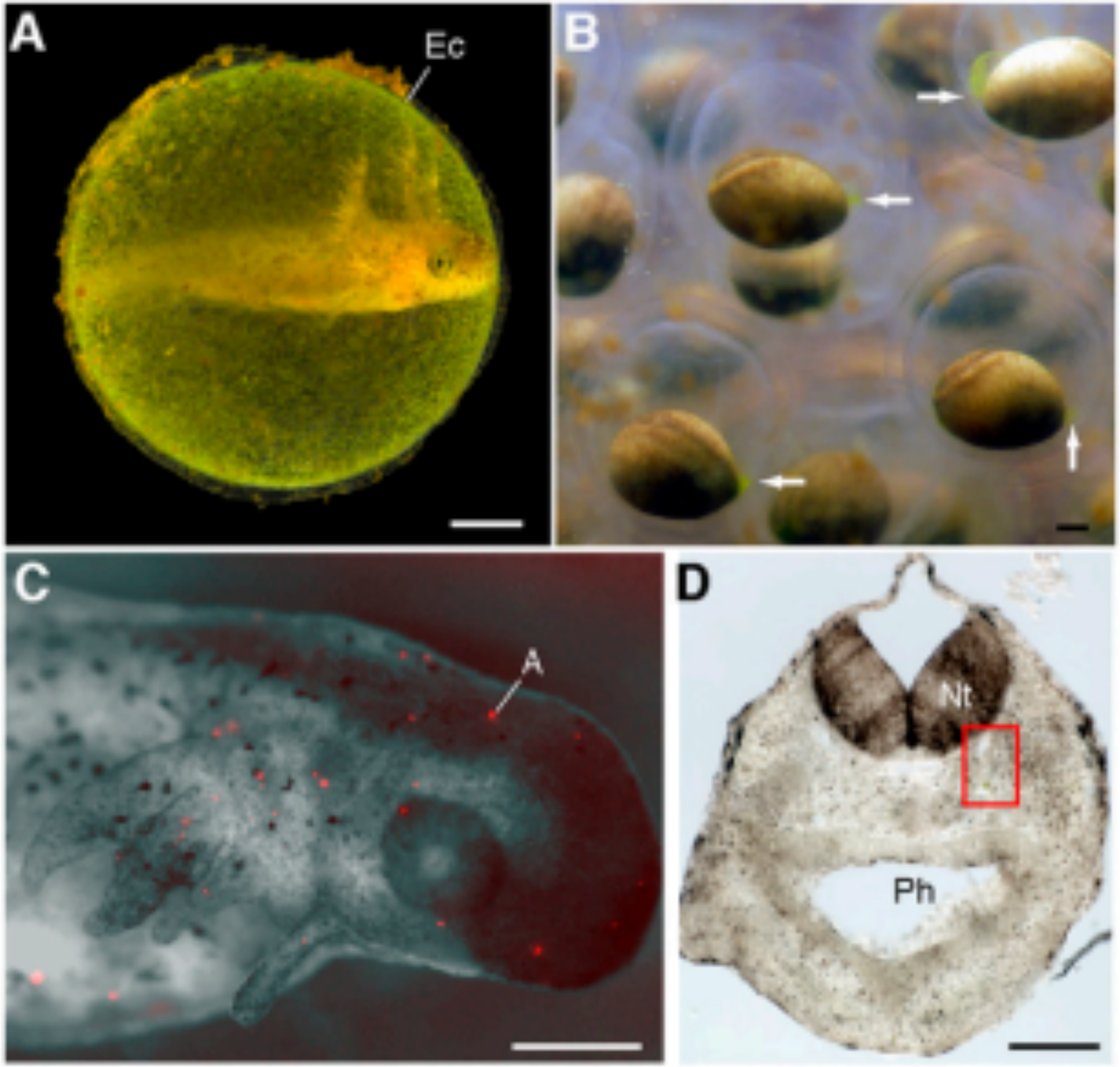


- Immature light organs have 2 pairs of ciliated appendages which facilitate infection of the organs with bacteria



Vibrio fischeri (Vf)
SENSING? PREPARATION?
Sea water
Attachment by 3h
Colonization (3h - 6h)
12,428
23
37
peptide degradation
chitin degradation
Aposymbiotic
Symbiotic
Transcriptomic analysis at 3 h
- Chemotactic gradient of chitobiose
- Priming of Vf
- Migration through the pores
Chitotriosidase
Chitin
Chitobiose
B
Progression of V. fischeri dominance
'Winnowing' away of nonsymbiotic bacteria by mucosal antimicrobial effector molecules
Adaptation during V. fischeri 'pause'
Upregulation of host chitotriosidase and priming of V. fischeri to sense chitobiose
C
Host establishment of a chitobiose gradient
Crypts
Migration of V. fischeri through the pores, following the chitobiose gradient established by chitotriosidase activity

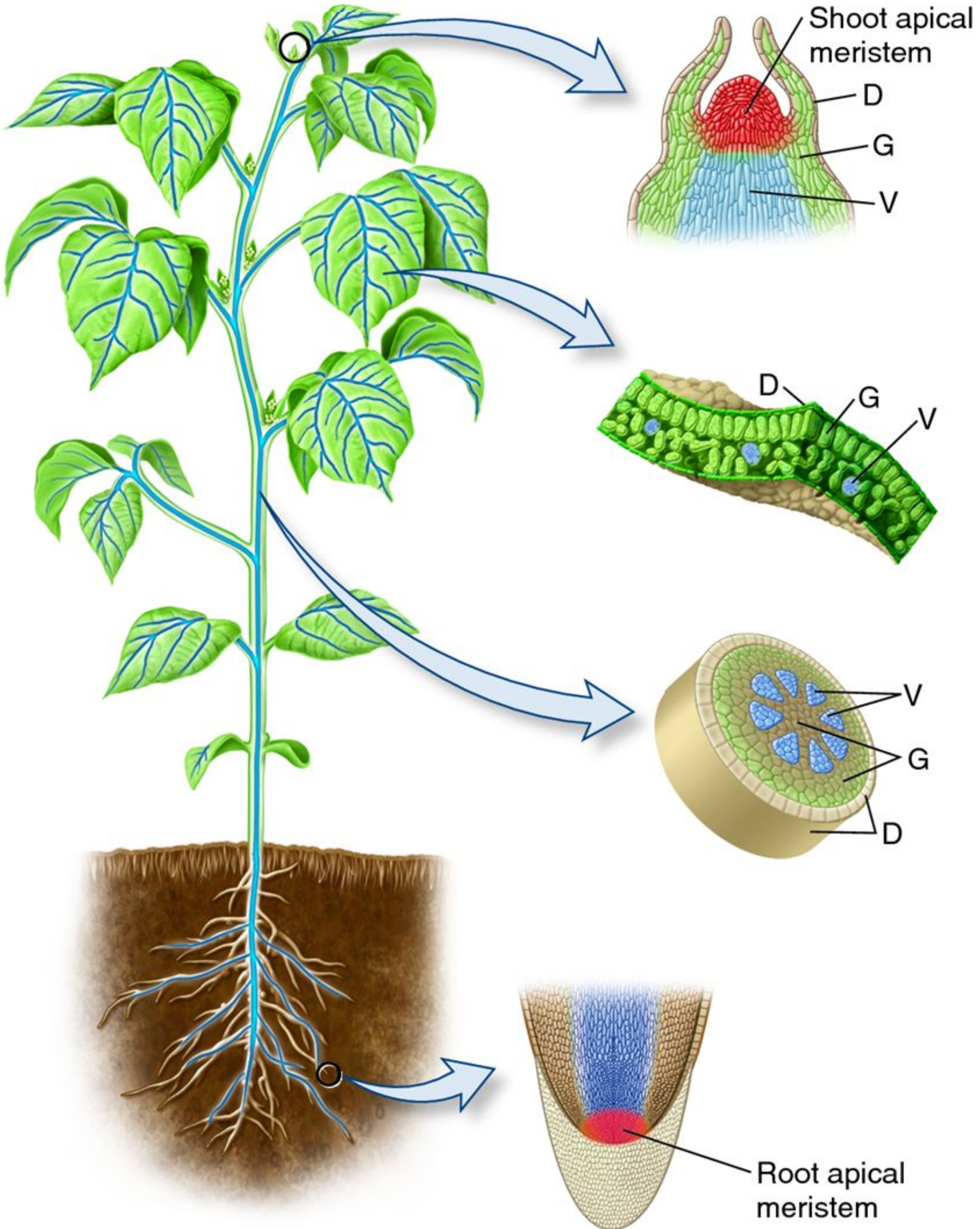

C
Apo
Sym
OMV


A
Ec
B
C
A
D
Nt
Ph

- Plants have an interesting development compared to animals
  - Root and shoot meristems are undifferentiated cells that divide throughout the plants lifetime

- Plants have a huge diversity of symbionts, most residing in the soil

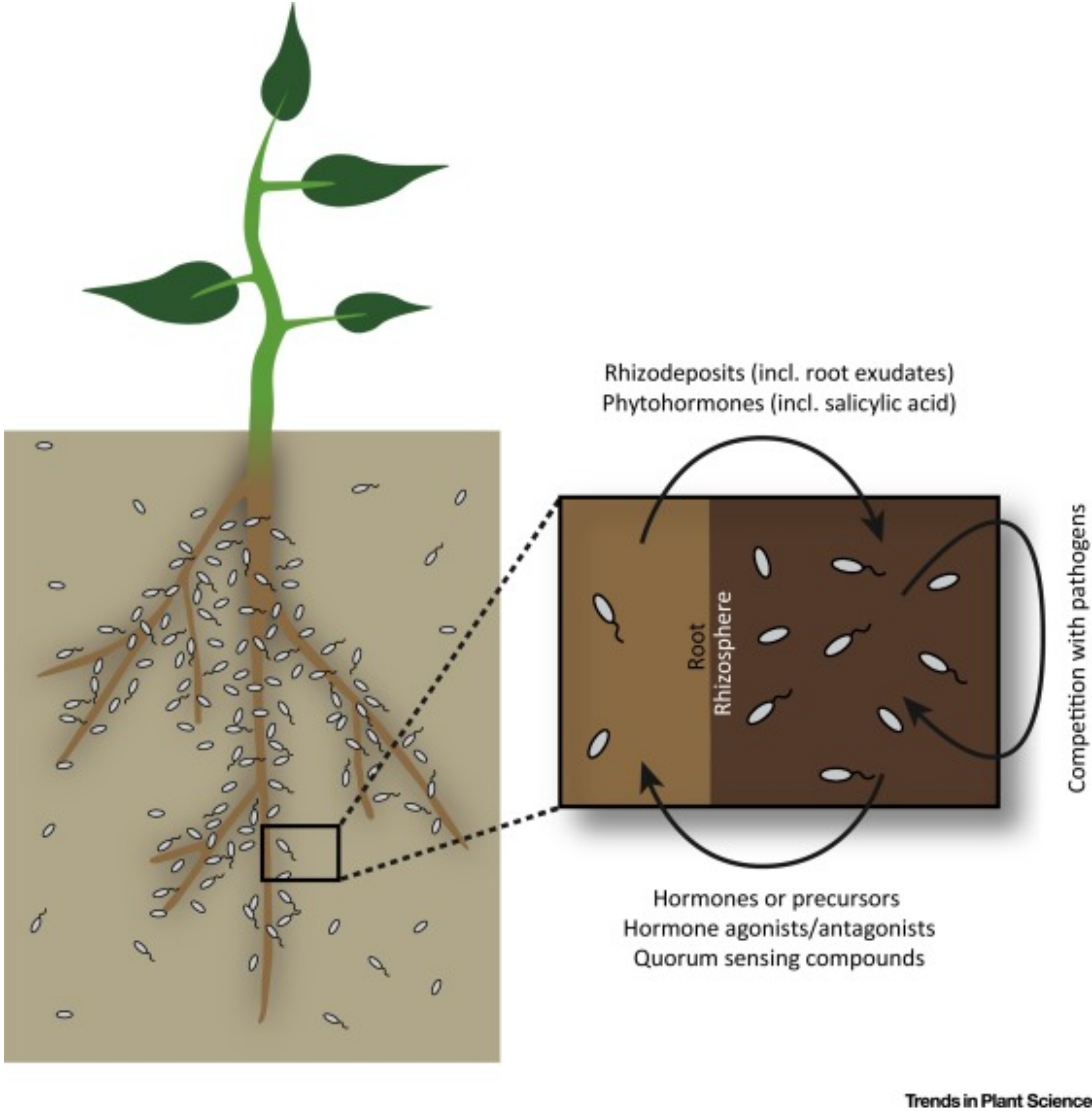
- Symbionts can influence the cell division rates within the meristematic zones and influence the root architecture as well as biomass of the plant




- These symbionts include Plant-Growth Promoting Rhizobacteria (PGPR)

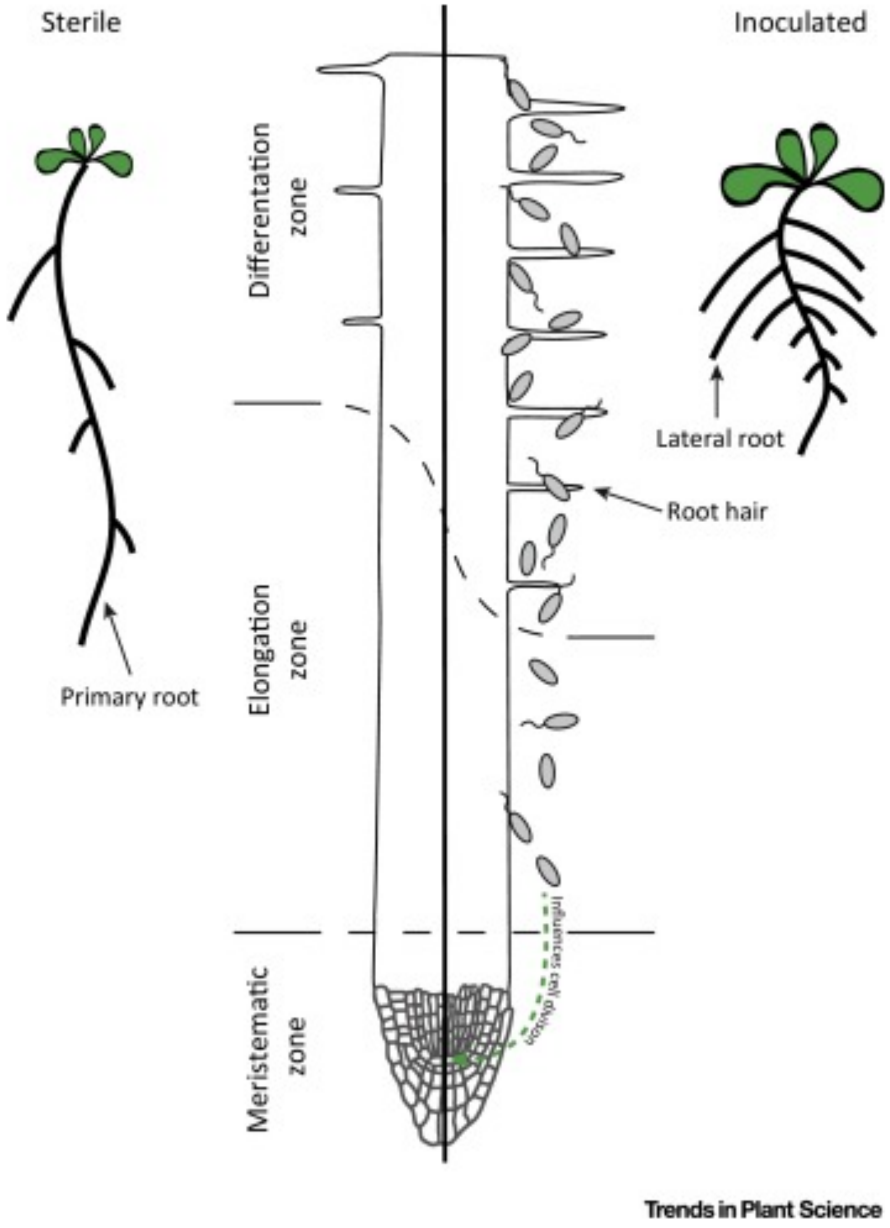
- Can secrete plant phytohormones or precursors

- Result in root architecture changes and biomass



- Primary root can split off into lateral roots, PGPR can cause an increase in primary root development, an increase in root hair, and differentiation closer to meristem

- PGPR produce phytohormones that may cause this increase in cell division and lateral root production



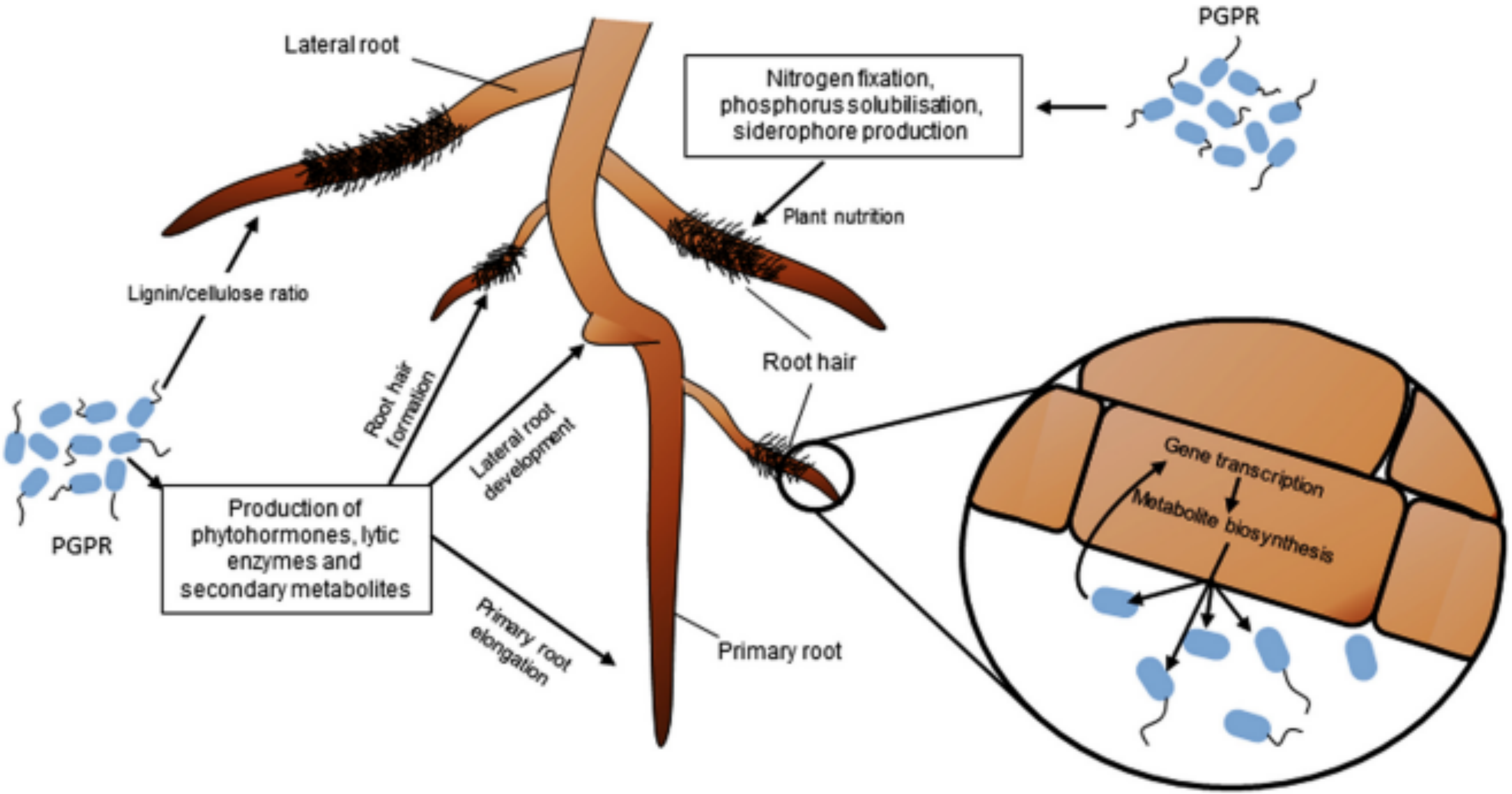
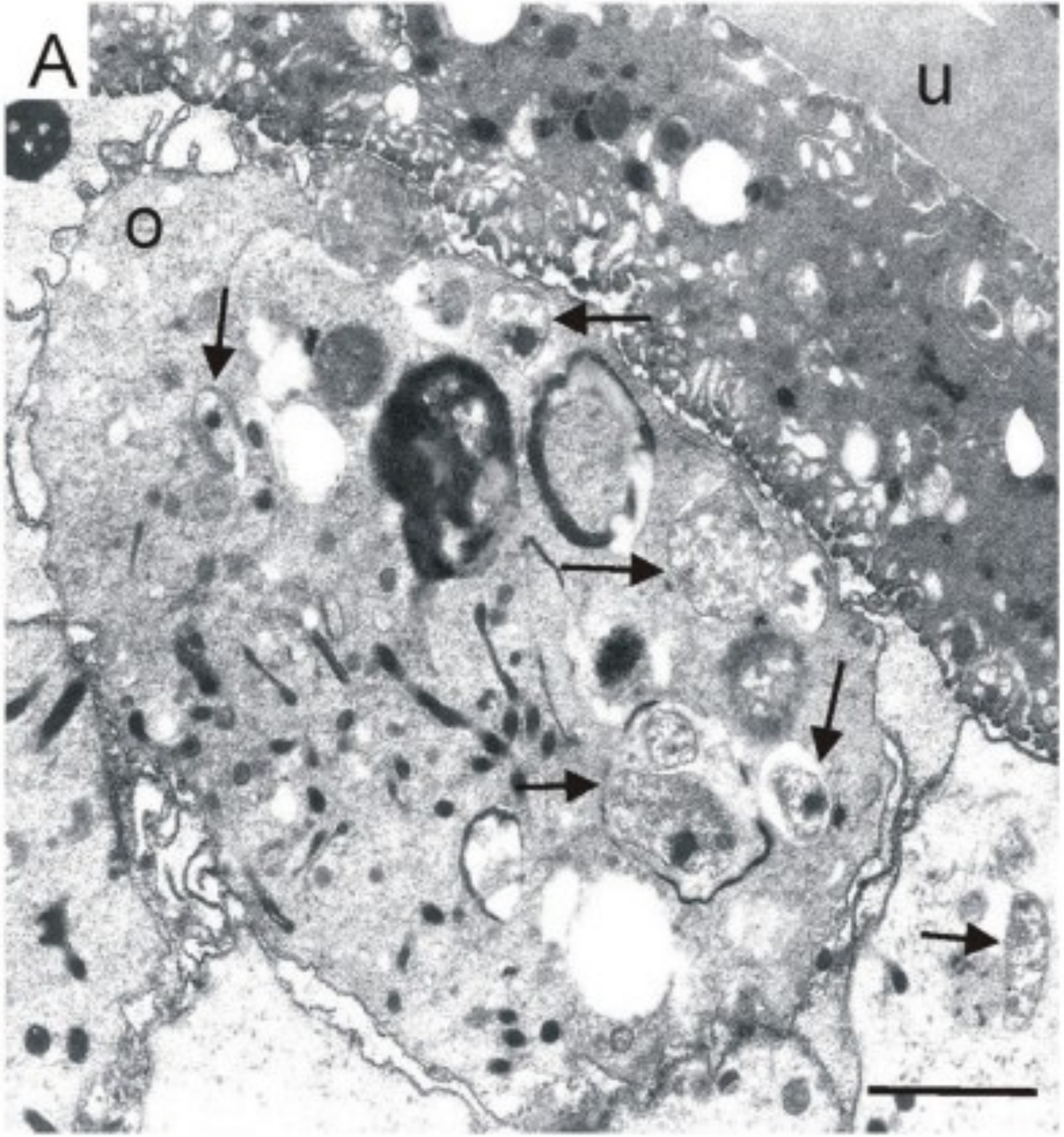


FIGURE 1 | Impact of phytostimulating PGPR on RSA, nutrient acquisition and root functioning. PGPR can modulate root development and growth through the production of phytohormones, secondary metabolites and enzymes. The most commonly observed effects are a reduction of the growth rate of primary root, and an increase of the number and length of lateral roots and root hairs. PGPR also influence plant nutrition via nitrogen fixation, solubilization of phosphorus, or siderophore production, and modify root physiology by changing gene transcription and metabolite biosynthesis in plant cells.

- Wolbachia are an endosymbiotic bacteria that infects arthropods, a high proportion of insects, and some nematodes
- Generally considered parasitic
- Wolbachia at arrows inside a nematode oocyte



- *Asobara tabida* is a braconid, parasitoid of D. melanogaster with an obligate symbiosis with *Wolbachia* bacteria

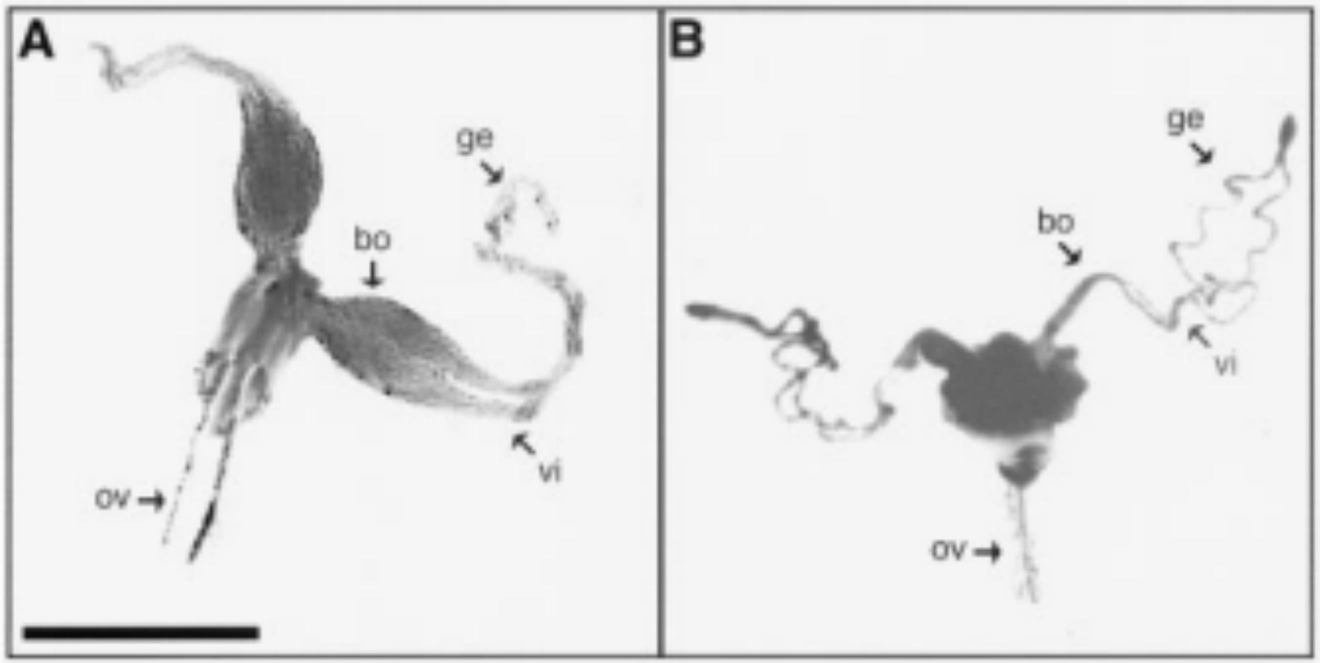
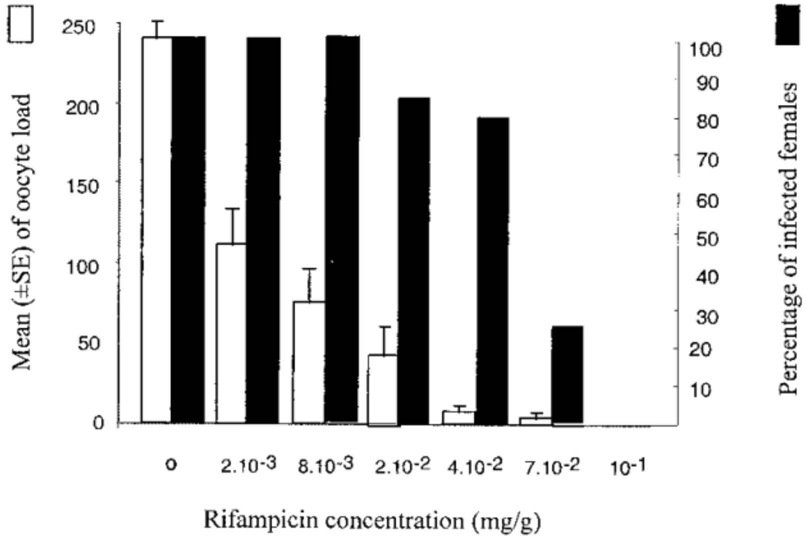
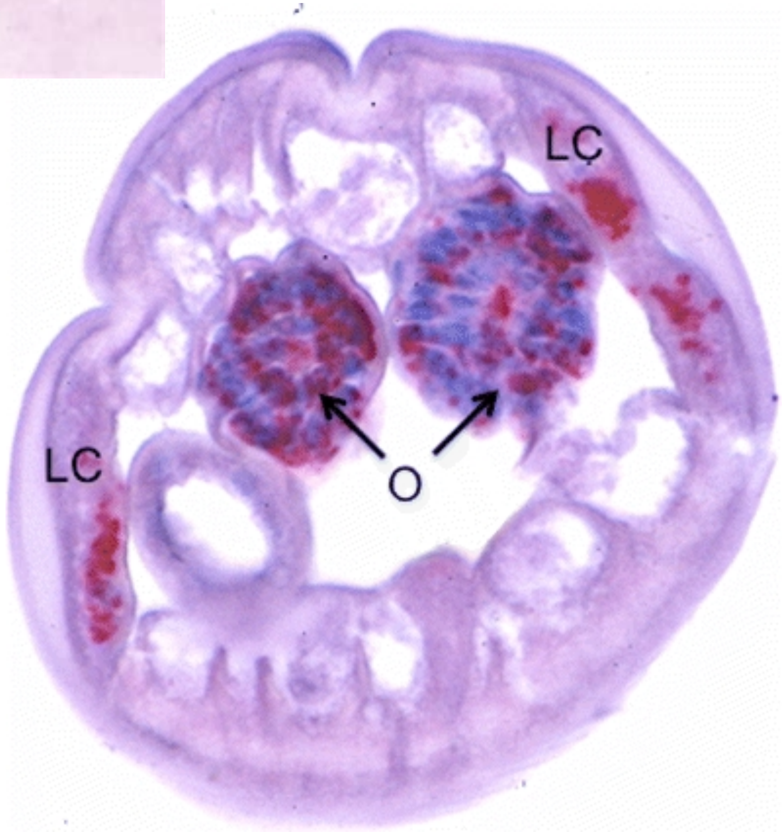
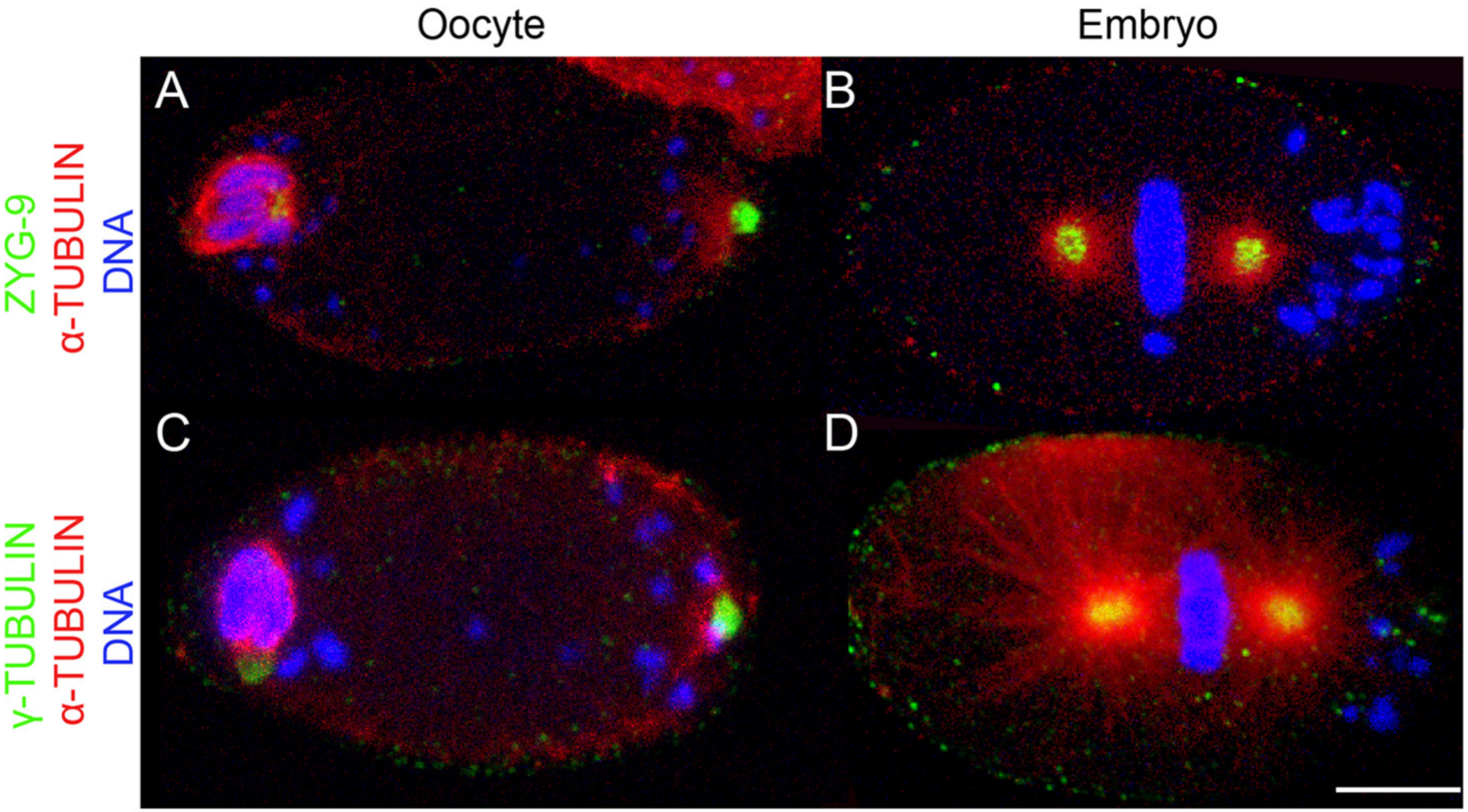


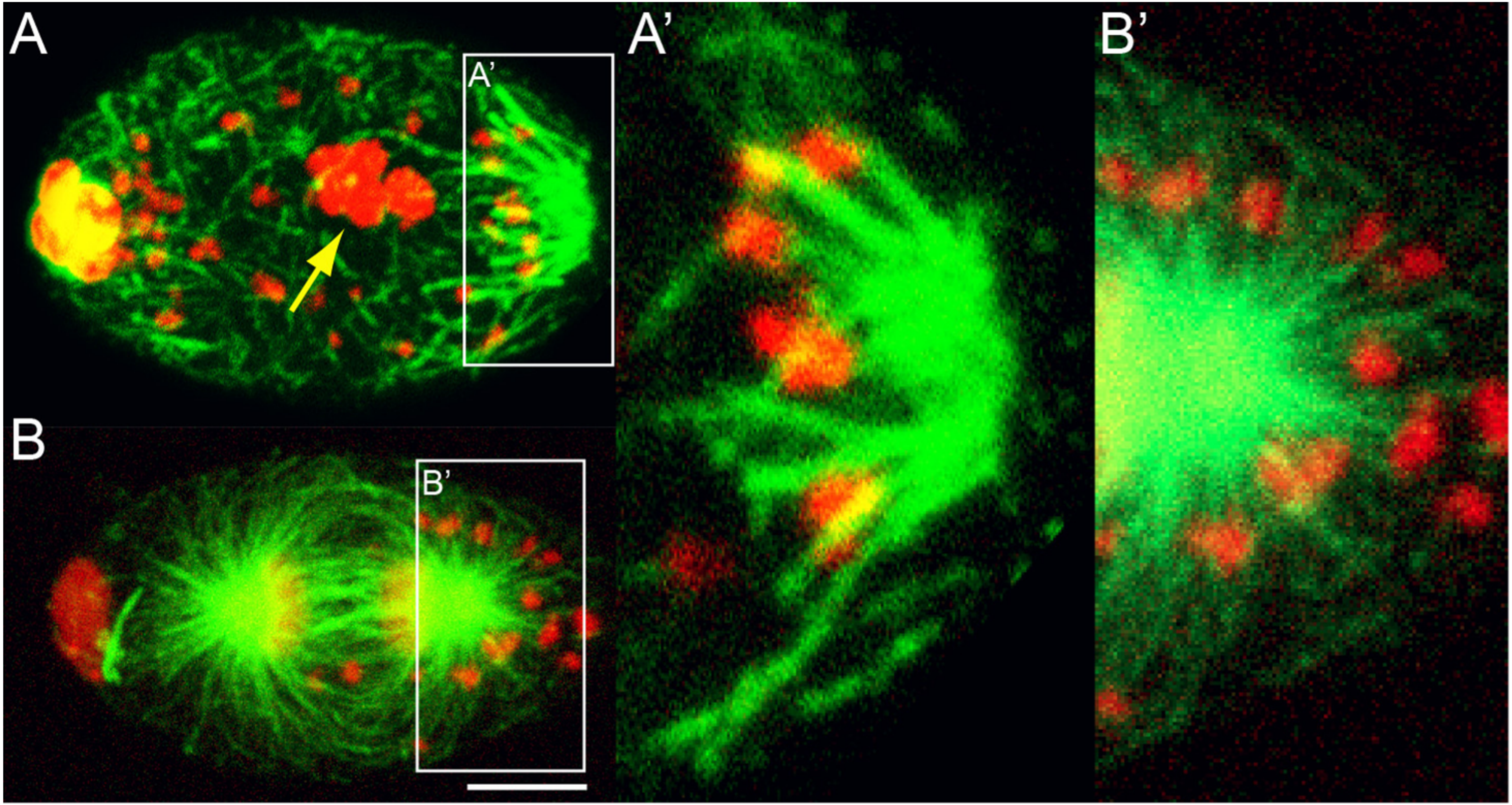
Fig. 1. Genital apparatus of *Asobara tabida* females at emergence. (*A*) Apparatus from untreated control female. Note the presence of numerous mature oocytes in the basal region of ovarioles (bo). (*B*) Apparatus from cured female (larval treatment, rifampicin, 2 mg/g). Note the total absence of oocyte in ovarioles. ge, germarium; ov, ovipositor; vi, vitellarium. [Bar = 1 mm (for *A* and *B*).]

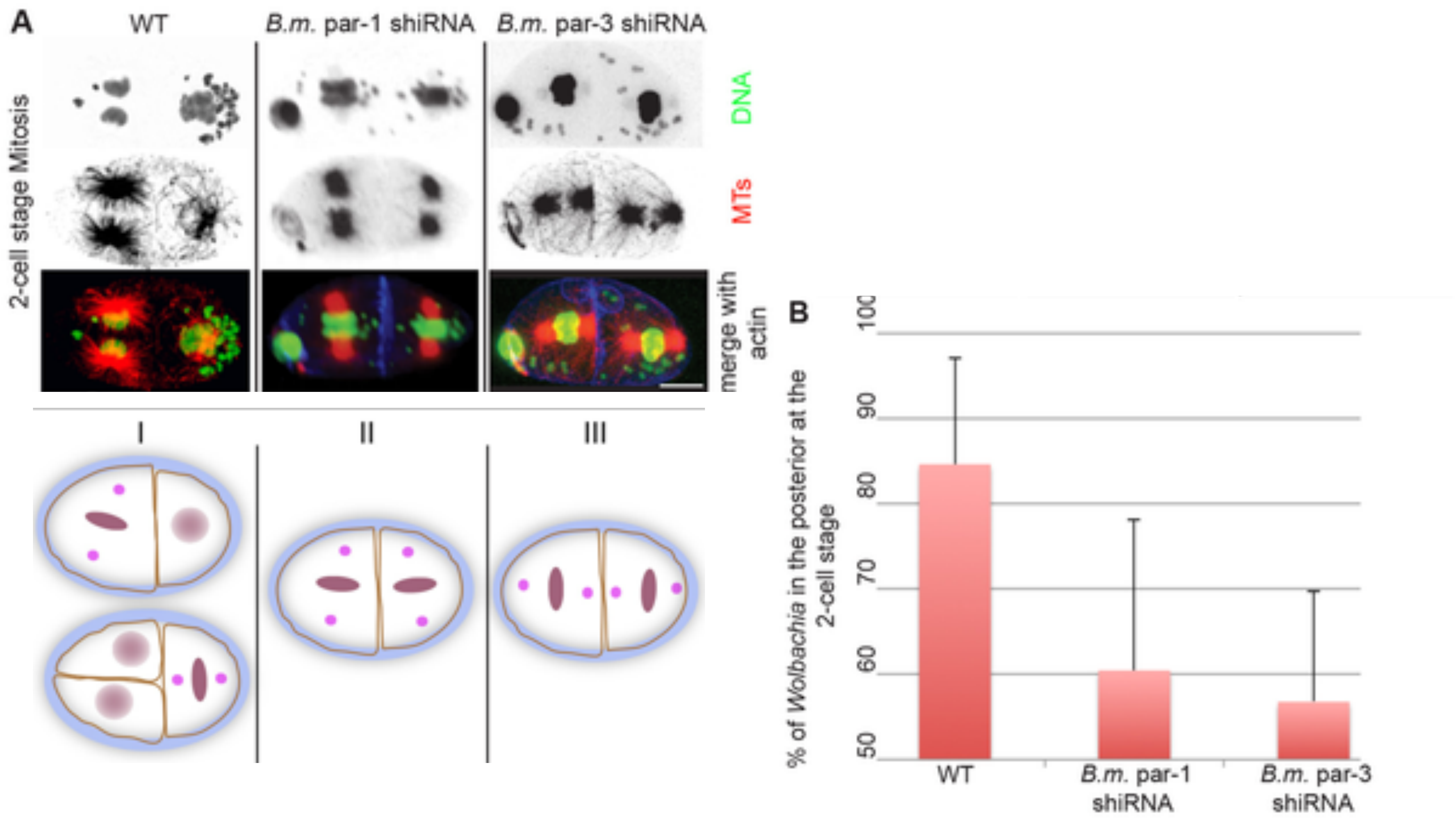


- *Brugia malayi* a filarial nematode that parasitizes humans and is spread through mosquitos
- Has symbiotic *Wolbachia* that are obligate, and removal leads to extensive apoptosis



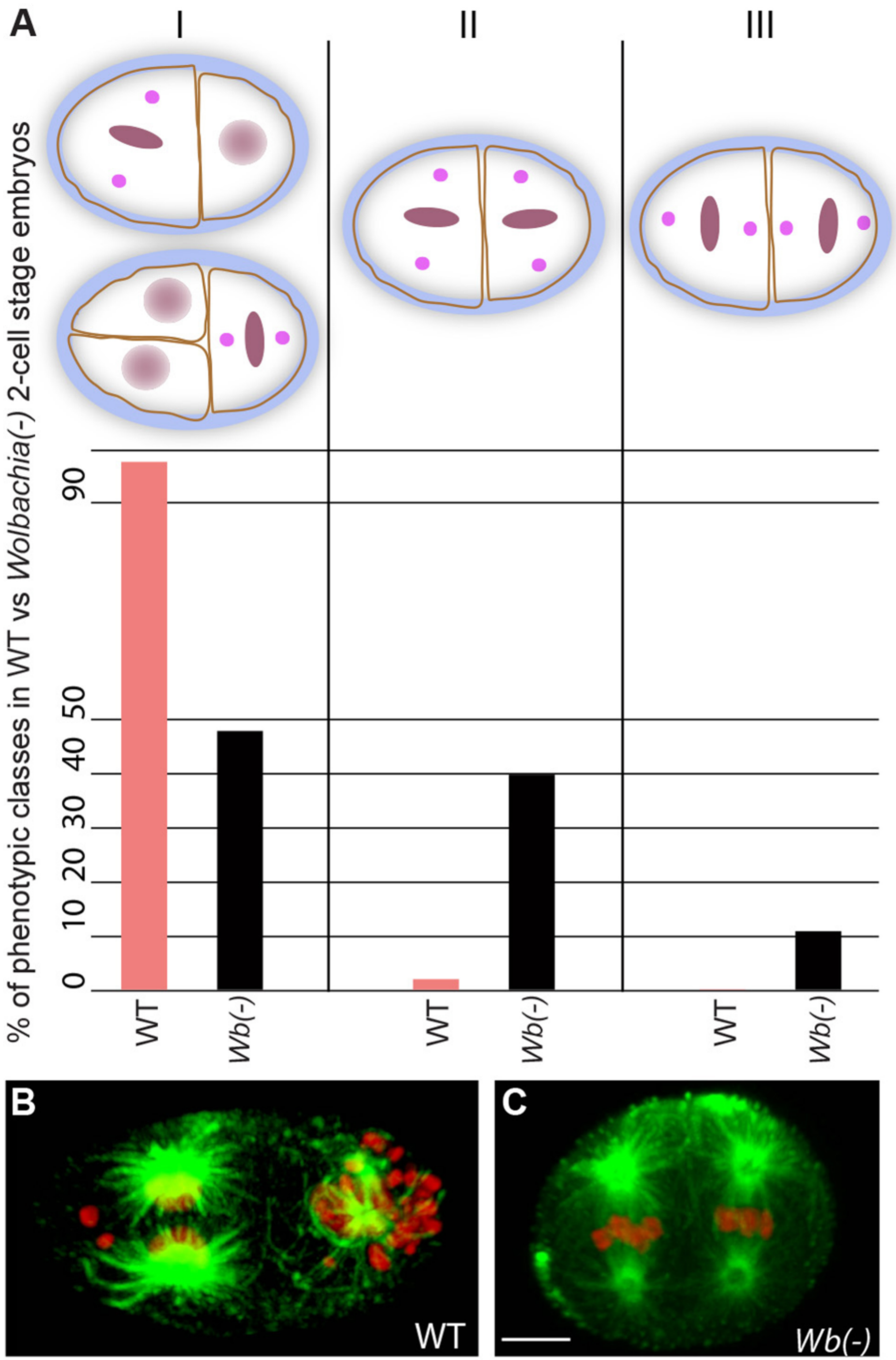

Oocyte
Embryo
A
B
C
D
ZYG-9
α-TUBULIN
DNA
γ-TUBULIN
α-TUBULIN
DNA


A
A’
A’
B
B’
B’

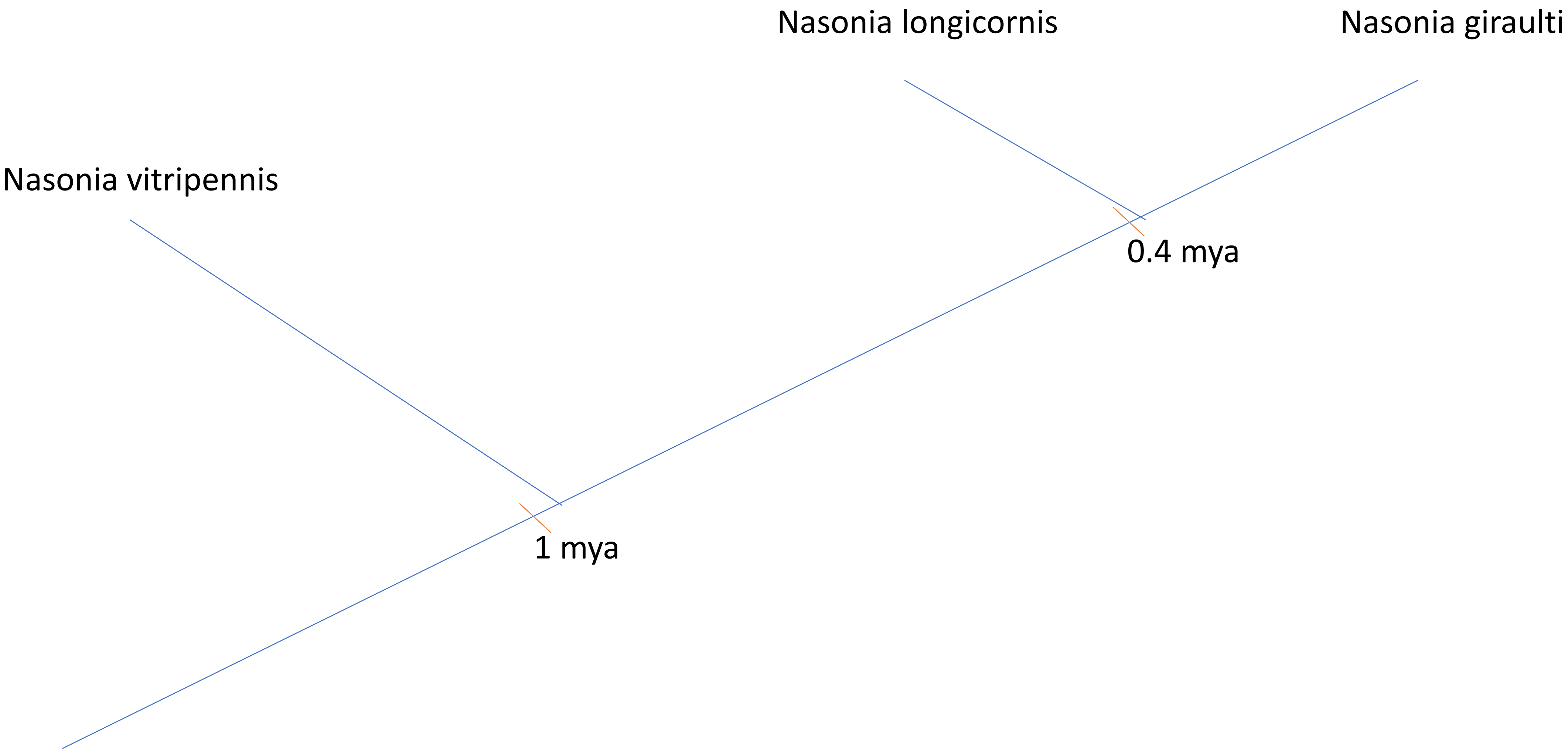


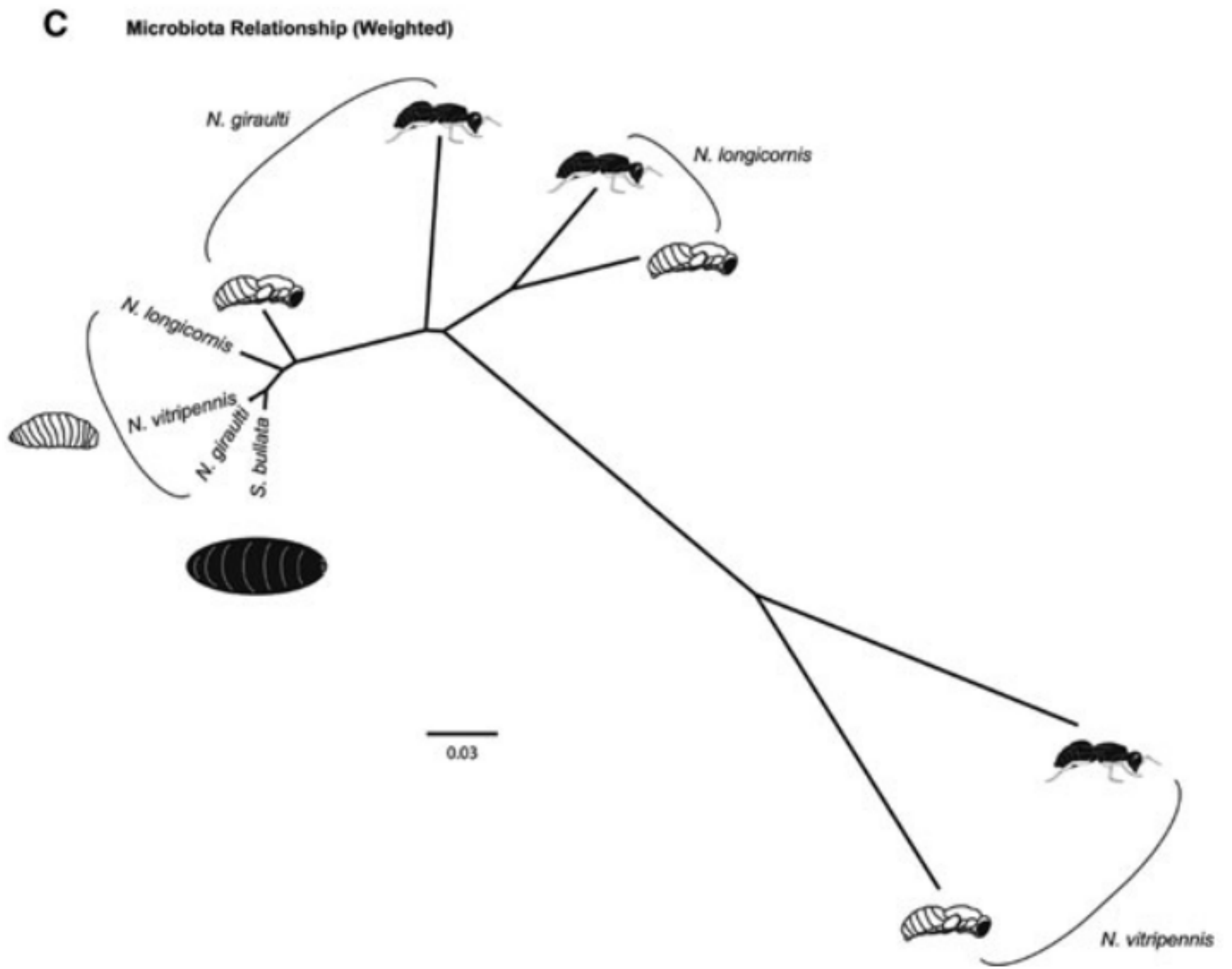
A
WT
B.m. par-1 shiRNA
B.m. par-3 shiRNA
2-cell stage Mitosis
DNA
MTs
merge with actin
I
II
III
B
% of Wolbachia in the posterior at the 2-cell stage
100
90
80
70
60
50
WT
B.m. par-1 shiRNA
B.m. par-3 shiRNA

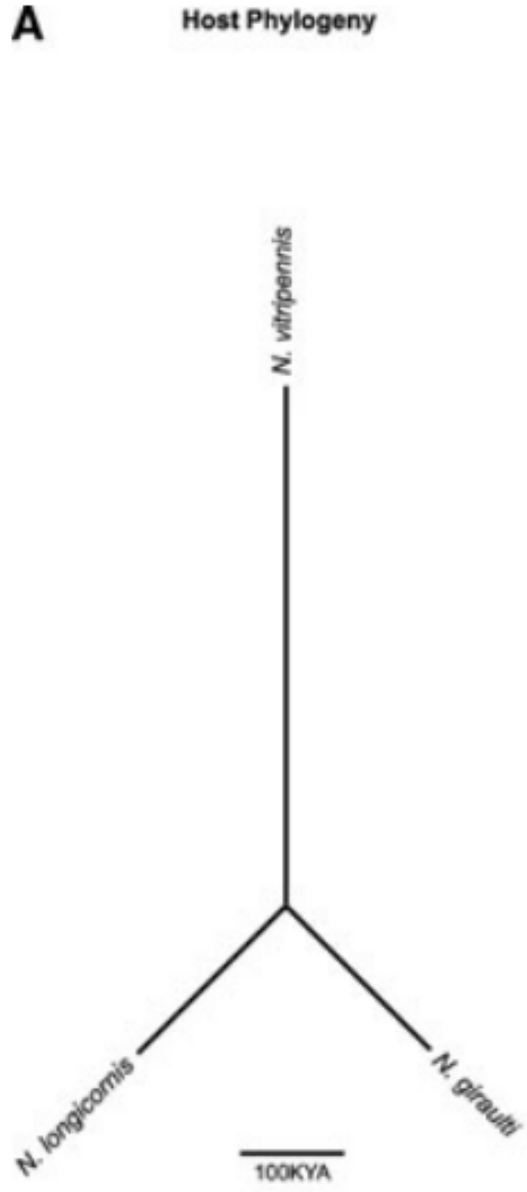
- Removal of Wolbachia resulted in very similar defects as the PAR knockdown genes
- Bacteria are necessary for normal embryonic development, and normal embryonic development is necessary for the successful colonization of the posterior pole
- Reciprocal dependence between the symbiont and host starting as early as the egg

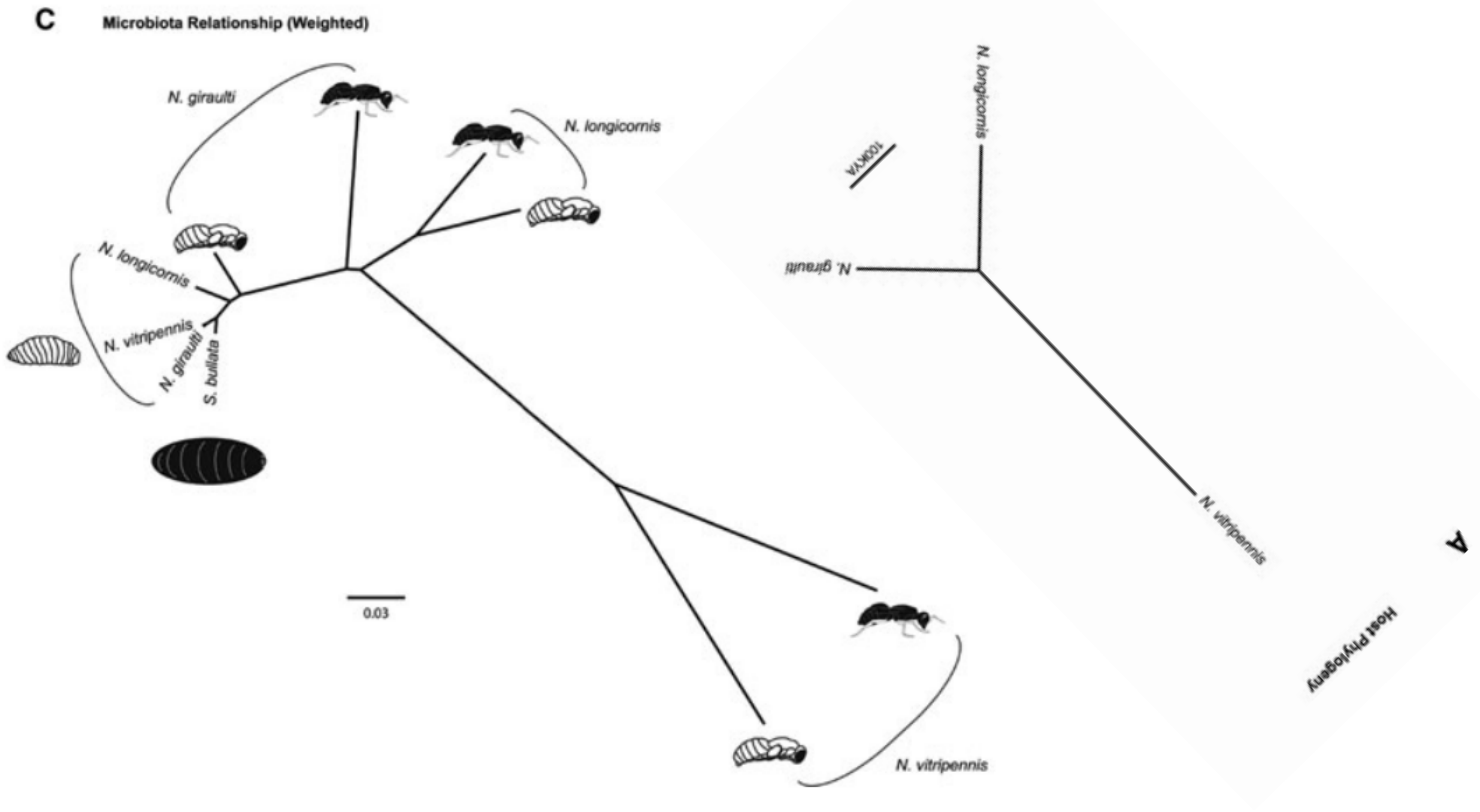


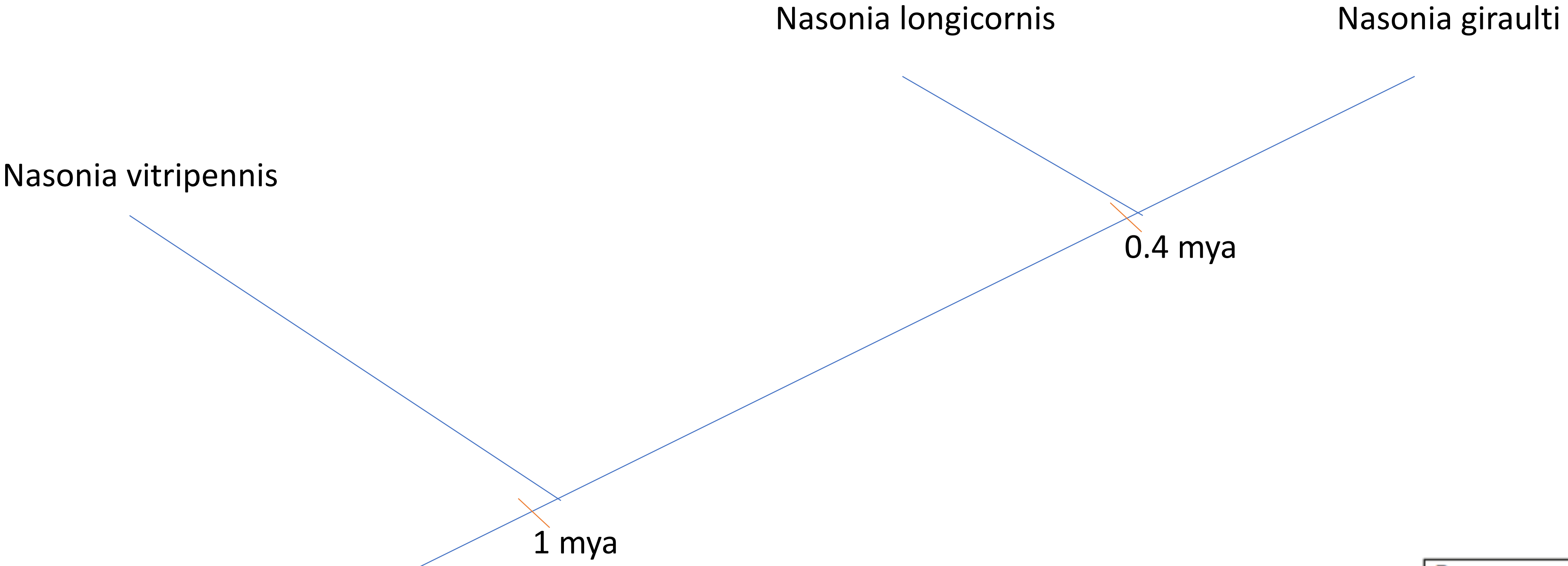
Symbionts Influencing Speciation


Nasonia longicornis
Nasonia giraulti
Nasonia vitripennis
0.4 mya
1 mya


C
Microbiota Relationship (Weighted)
N. giraulti
N. longicornis
N. longicornis
N. vitripennis
N. giraulti
S. bullata
0.03
N. vitripennis

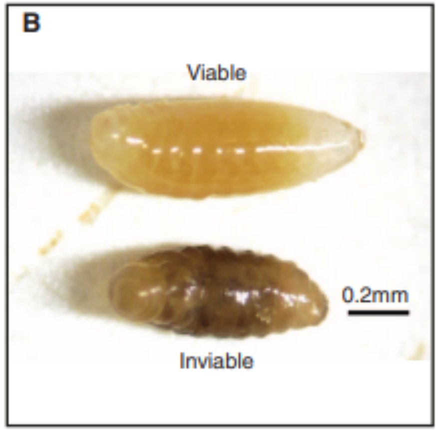

A
Host Phylogeny
N. vitripennis
N. longicornis
N. giraulti
100KYA

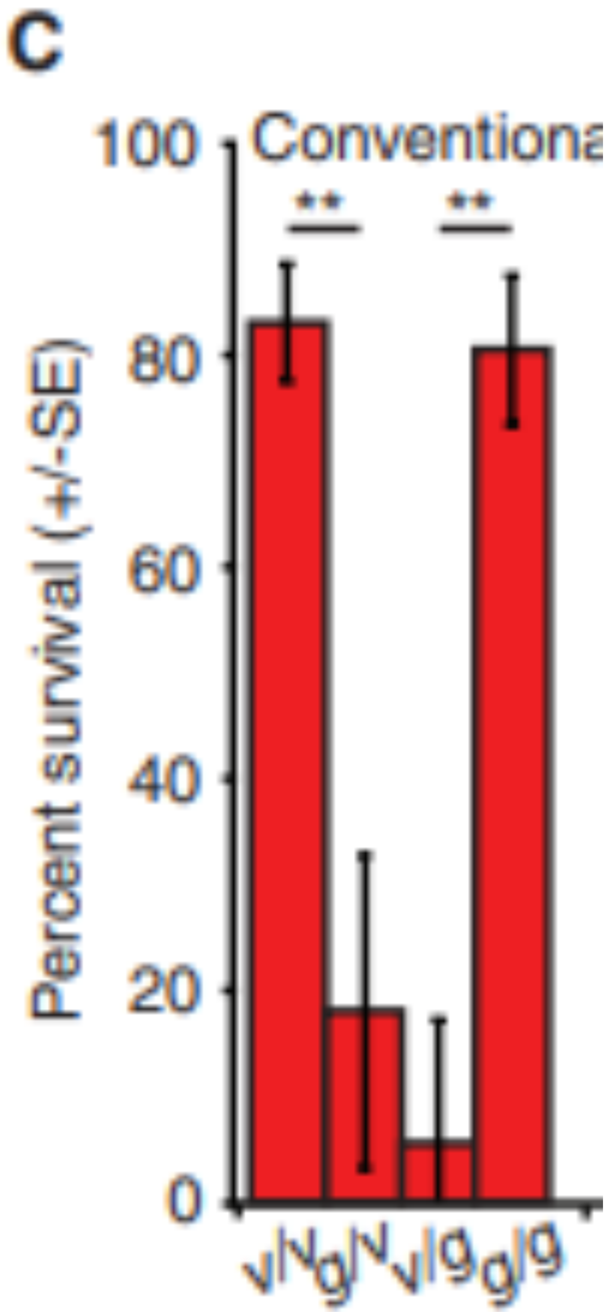

C
Microbiota Relationship (Weighted)
N. giraulti
N. longicornis
N. longicornis
N. vitripennis
N. giraulti
S. bullata
0.03
N. vitripennis
A
Host Phylogeny
100KYA
N. longicornis
N. giraulti
N. vitripennis

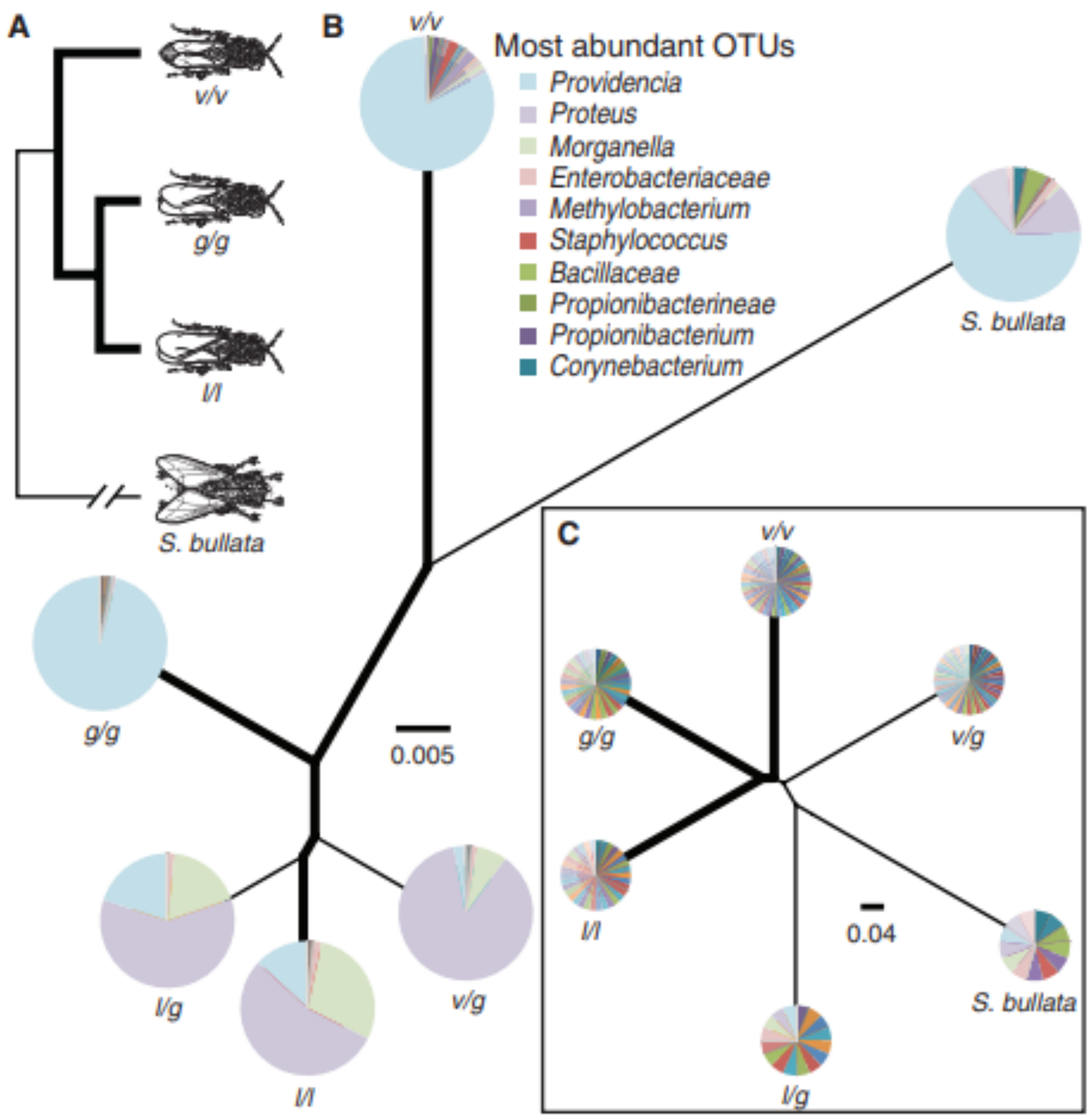


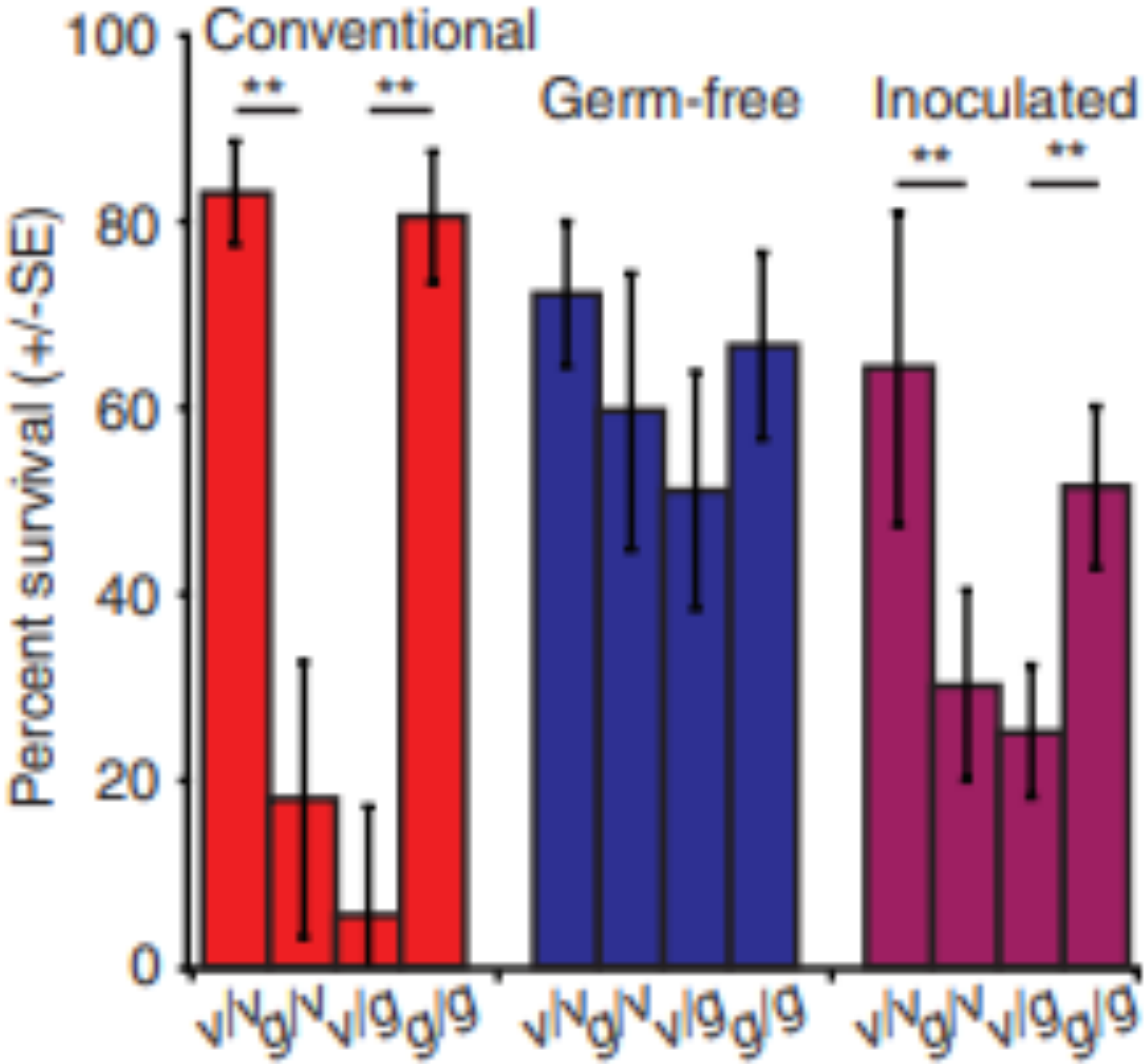
N. vitripennis and N. longicornis / giraulti crosses have 90% lethality rate

N. longicornis and N. giraulti hyrbids have around 8% lethality rate




C
Conventiona
100
80
60
40
20
0
Percent survival (+/-SE)
**
**
v|N
g|N
v|g
g|g


A
v/v
g/g
l/l
S. bullata
B
v/v
Most abundant OTUs
Providencia
Proteus
Morganella
Enterobacteriaceae
Methylobacterium
Staphylococcus
Bacillaceae
Propionibacterineae
Propionibacterium
Corynebacterium
S. bullata
g/g
0.005
l/g
l/l
v/g
C
v/v
g/g
v/g
l/l
0.04
S. bullata
l/g

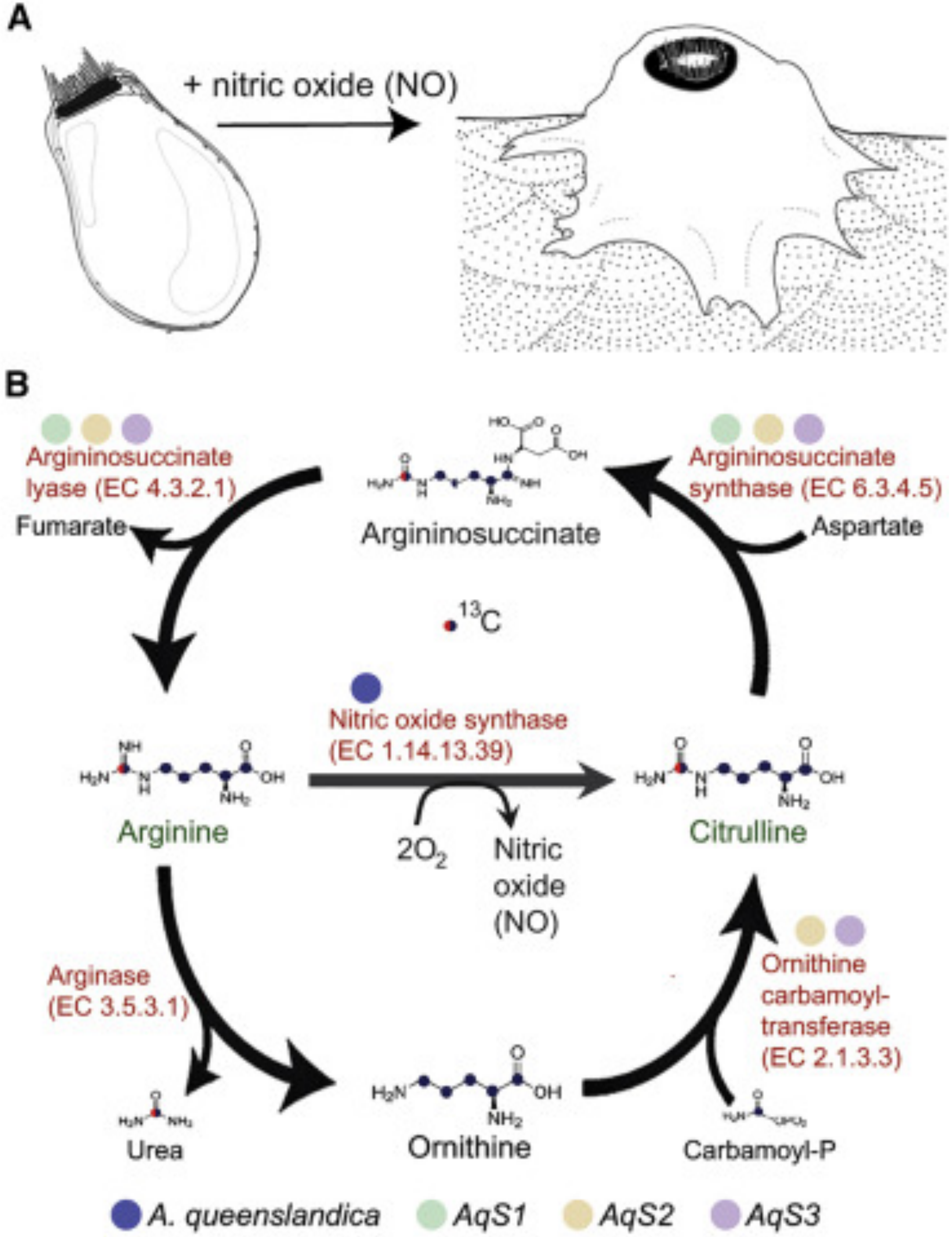


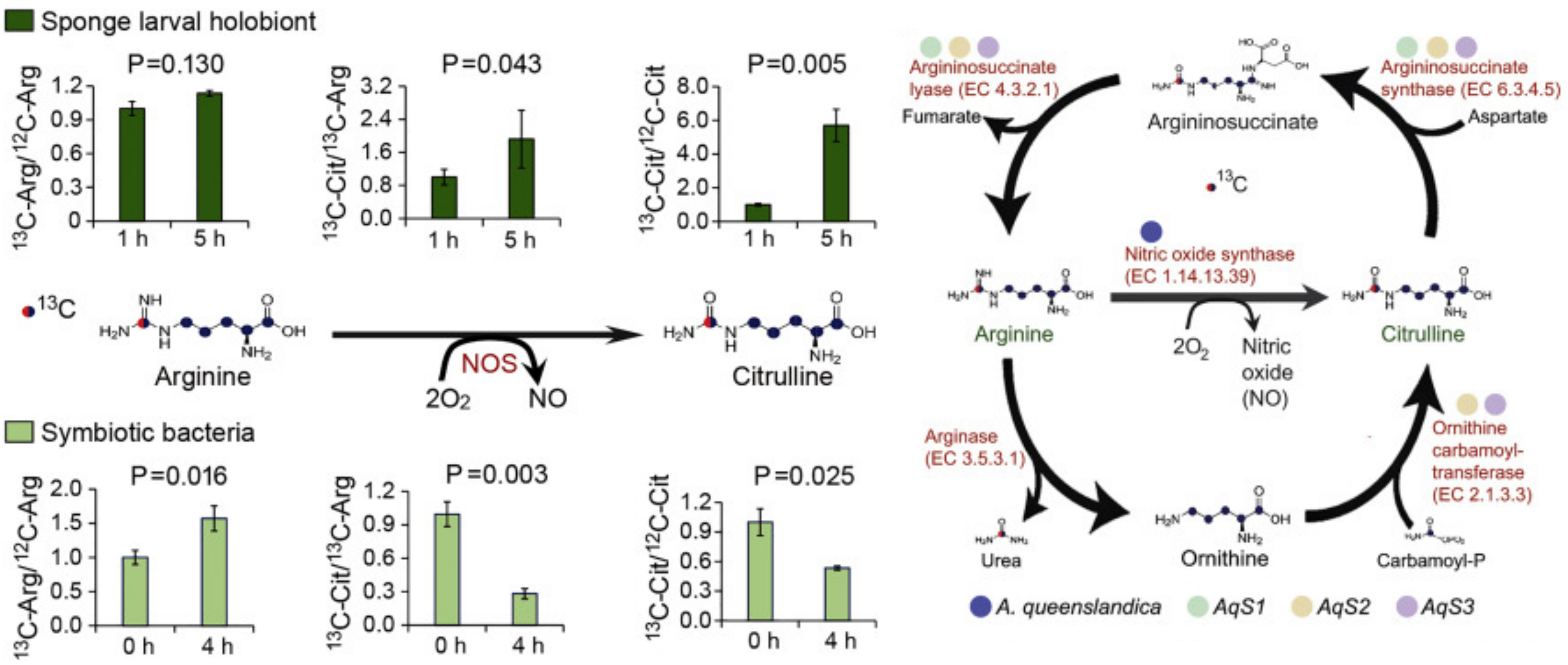
Conventional
Germ-free
Inoculated
**
**
**
**
Percent survival (+/-SE)
100
80
60
40
20
0
V|N
g|N
V|g
g|g
V|N
g|N
V|g
g|g
V|N
g|N
V|g
g|g

- A. Queenslandica needs arginine to biosynthesis NO for signalling pathways involved in development, primarily metamorphosis and larval settlement
- Genome is missing two synthetases involved in converting citrulline to arginine

- Adult can get arginine from feeding, but larvae are non-feeding so the two main sources of arginine would be from seawater absorption or symbionts.

- A. Queenslandica harbors a stable and low-complexity bacterial community dominated by 3 species, AqS1,2,3 that can biosynthesize arginine from host-produced citrulline



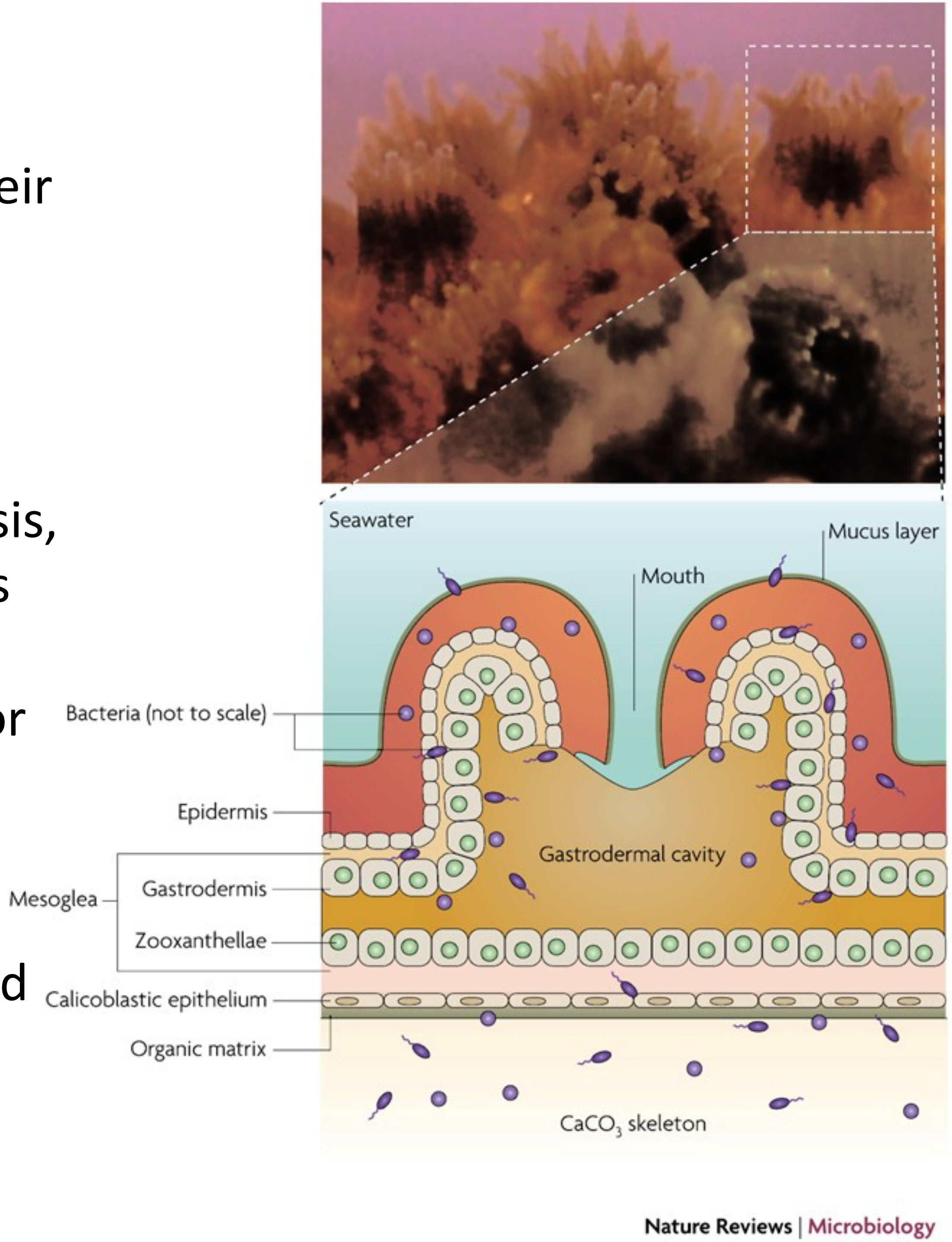

Sponge larval holobiont
P=0.130
P=0.043
P=0.005
^{13}C-Arg/^{12}C-Arg
^{13}C-Cit/^{13}C-Arg
^{13}C-Cit/^{12}C-Cit
1 h
5 h
^{13}C
Arginine
NOS
2O$_2$
NO
Citrulline
Symbiotic bacteria
P=0.016
P=0.003
P=0.025
0 h
4 h
Argininosuccinate lyase (EC 4.3.2.1)
Fumarate
Argininosuccinate
Argininosuccinate synthase (EC 6.3.4.5)
Aspartate
Nitric oxide synthase (EC 1.14.13.39)
Arginine
Citrulline
2O$_2$
Nitric oxide (NO)
Arginase (EC 3.5.3.1)
Ornithine carbamoyl-transferase (EC 2.1.3.3)
Urea
Ornithine
Carbamoyl-P
A. queenslandica
AqS1
AqS2
AqS3

Corals are associated with microbes across their anatomy → mucus layer, in tissues, and in skeleton

These symbionts can benefit the coral host through various mechanisms → photosynthesis, nitrogen fixation, production of antimicrobials

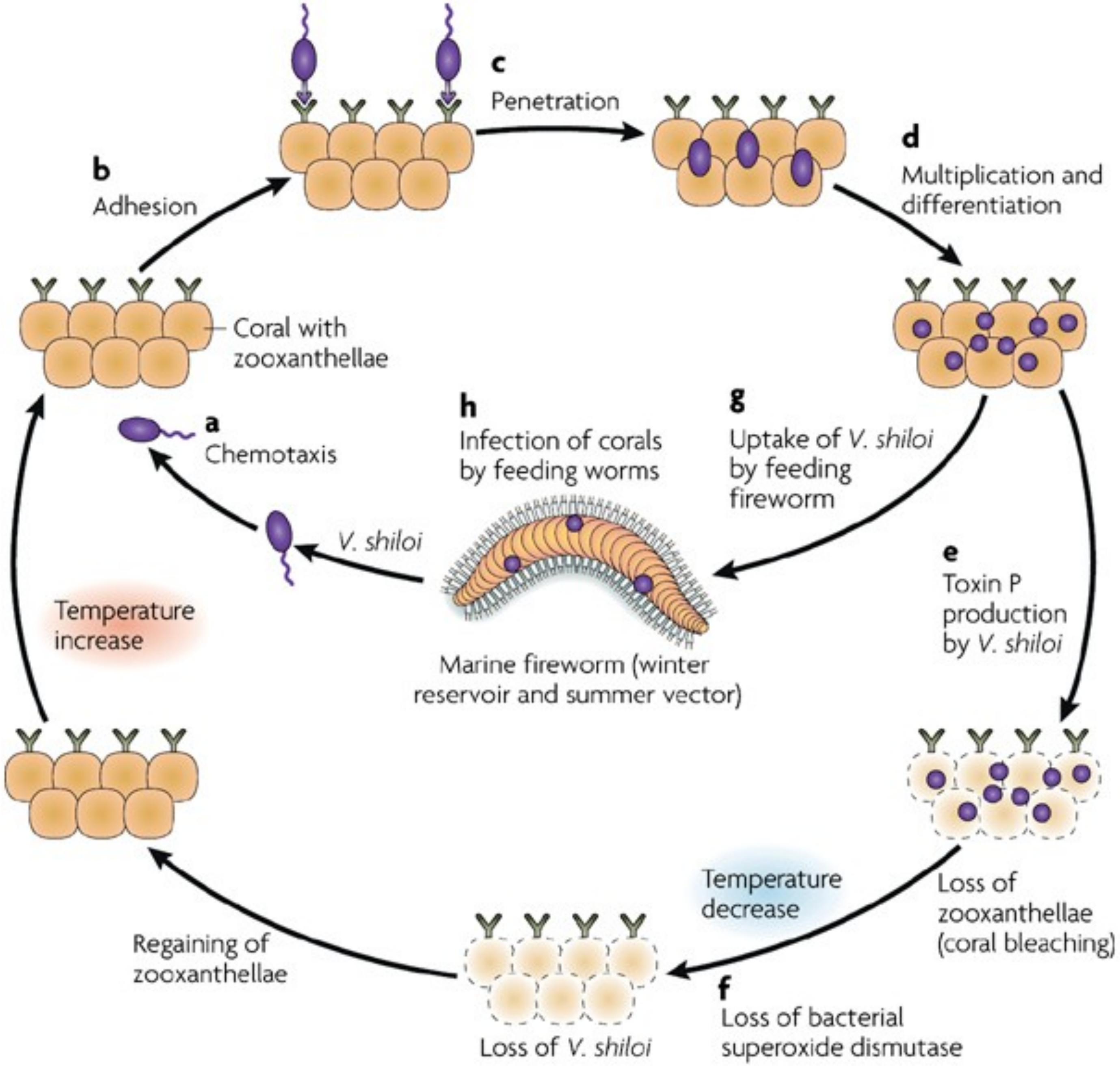
Microbes can also be the cause of bleaching or coral diseases

These coral associated microbes undergo rapid changes when environmental conditions are altered



- Coral has an innate immune system but lacks an adaptive immune system

- Can become resistant to certain microbes or diseases



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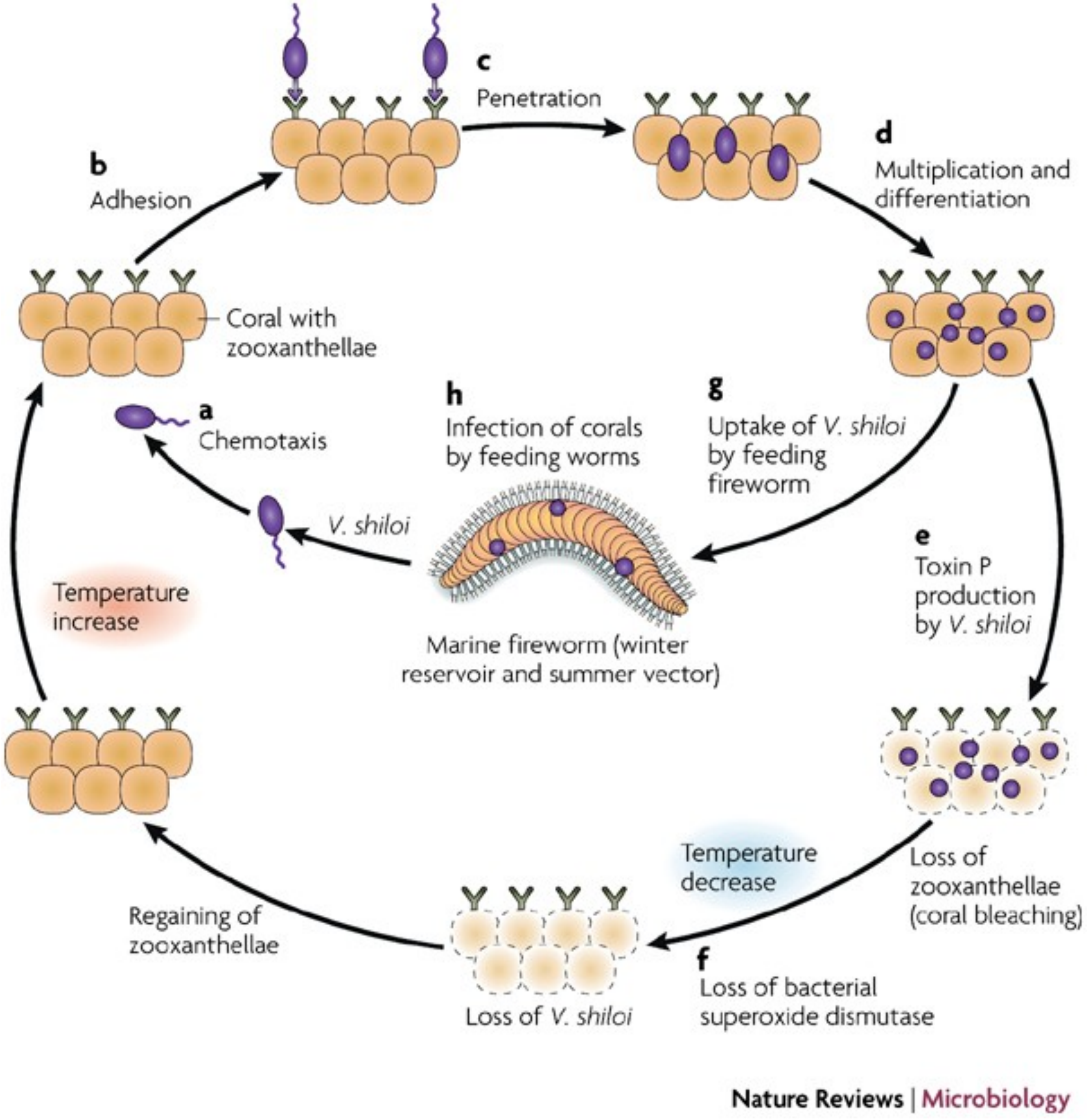
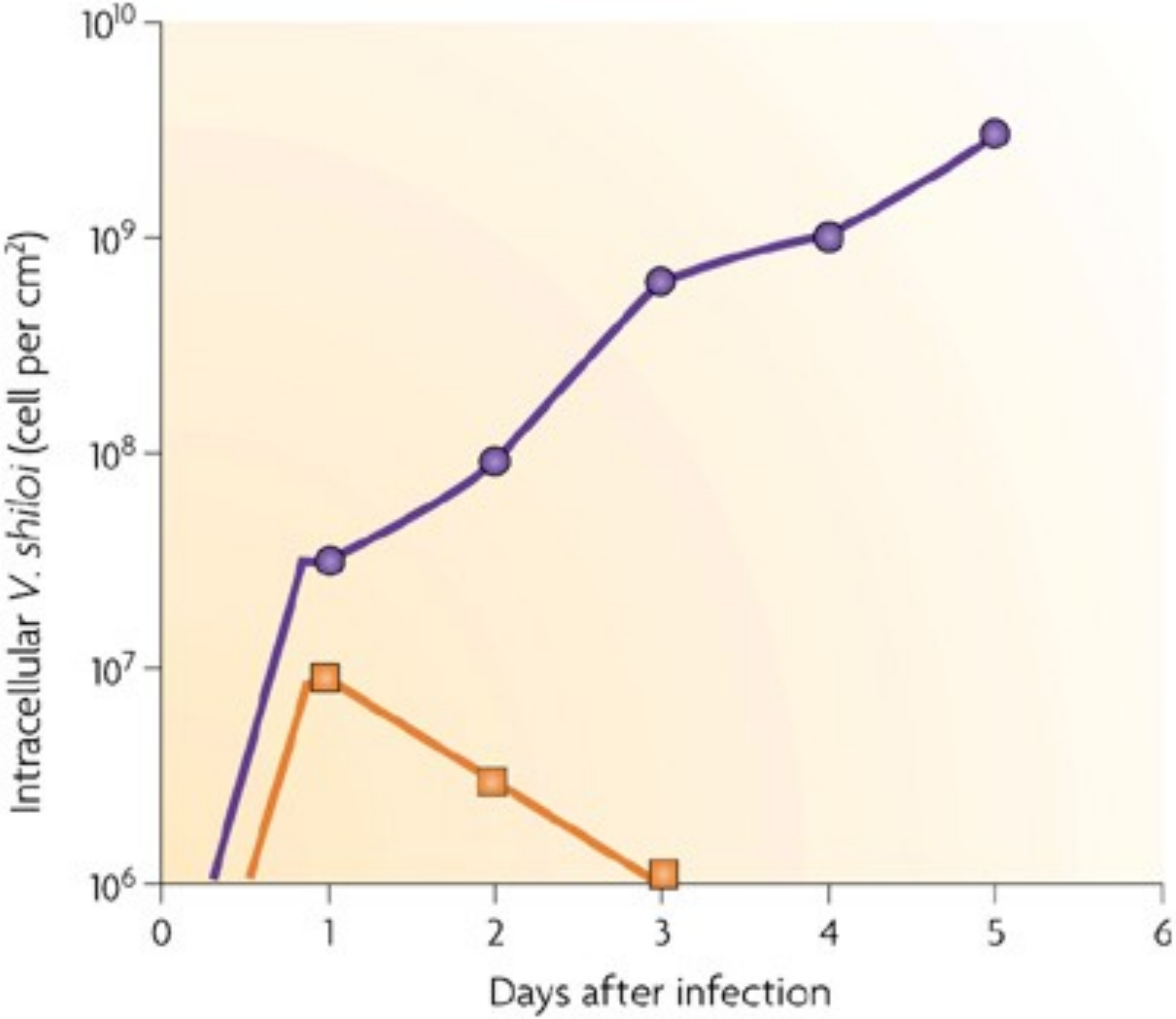
- From 1994 to 2002 bacterial bleaching of the coral O. patagonica occurred every summer in the eastern mediterannean, and V. shiloi was repeatedly isolated from bleached corals and caused bleaching in health corals in aquaria experiments



- Purple are coral prior to 2003, orange are after

- V. shiloi can still infect the corals, but the number of bacteria cells drops drastically after 1 day



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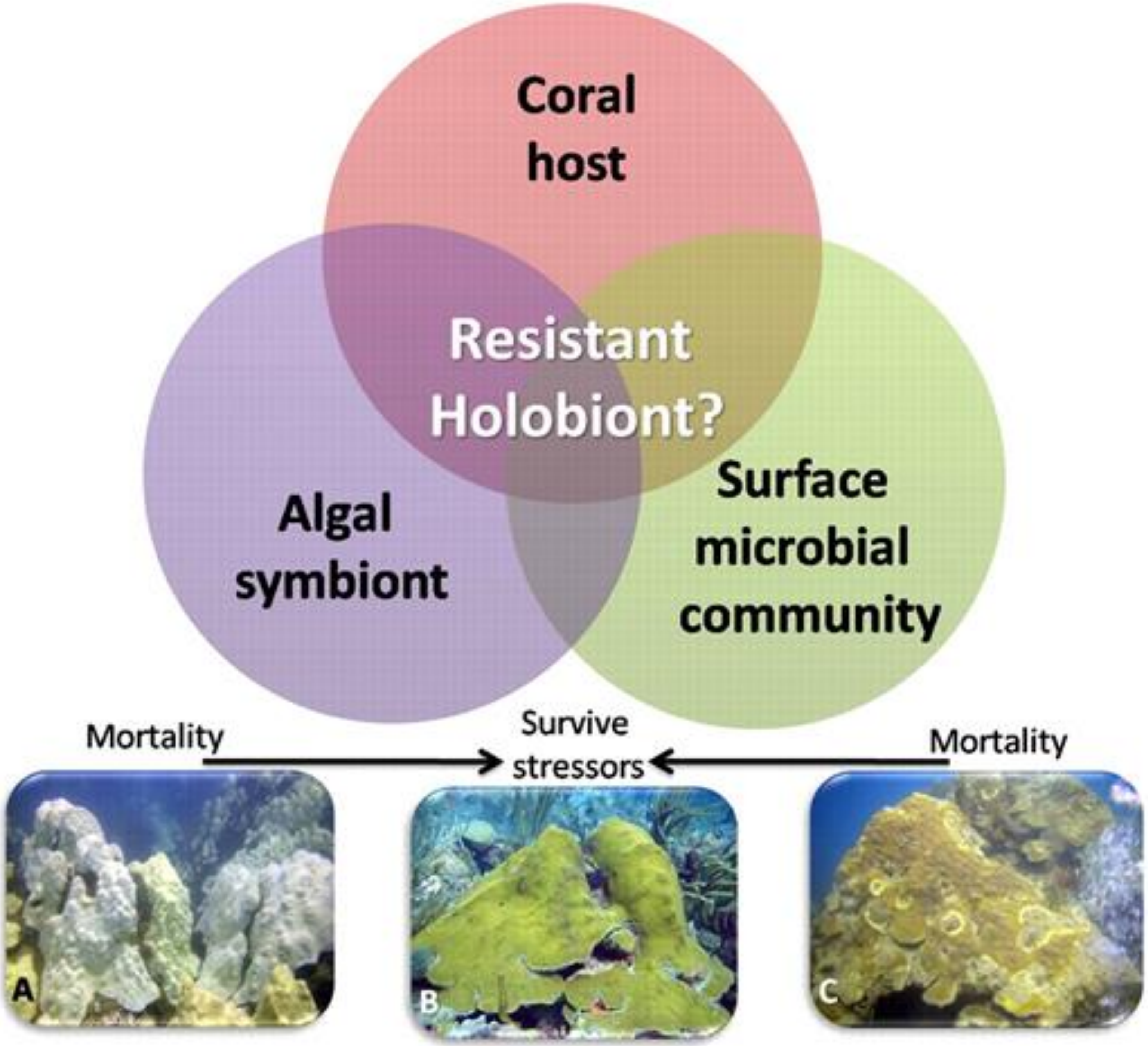

Coral host
Resistant Holobiont?
Algal symbiont
Surface microbial community
Mortality
Survive stressors
Mortality
A
B
C

Hologenome theory of Evolution

- The holobiont, and the collective genome, the hologenome, should be considered a single unit in evolution

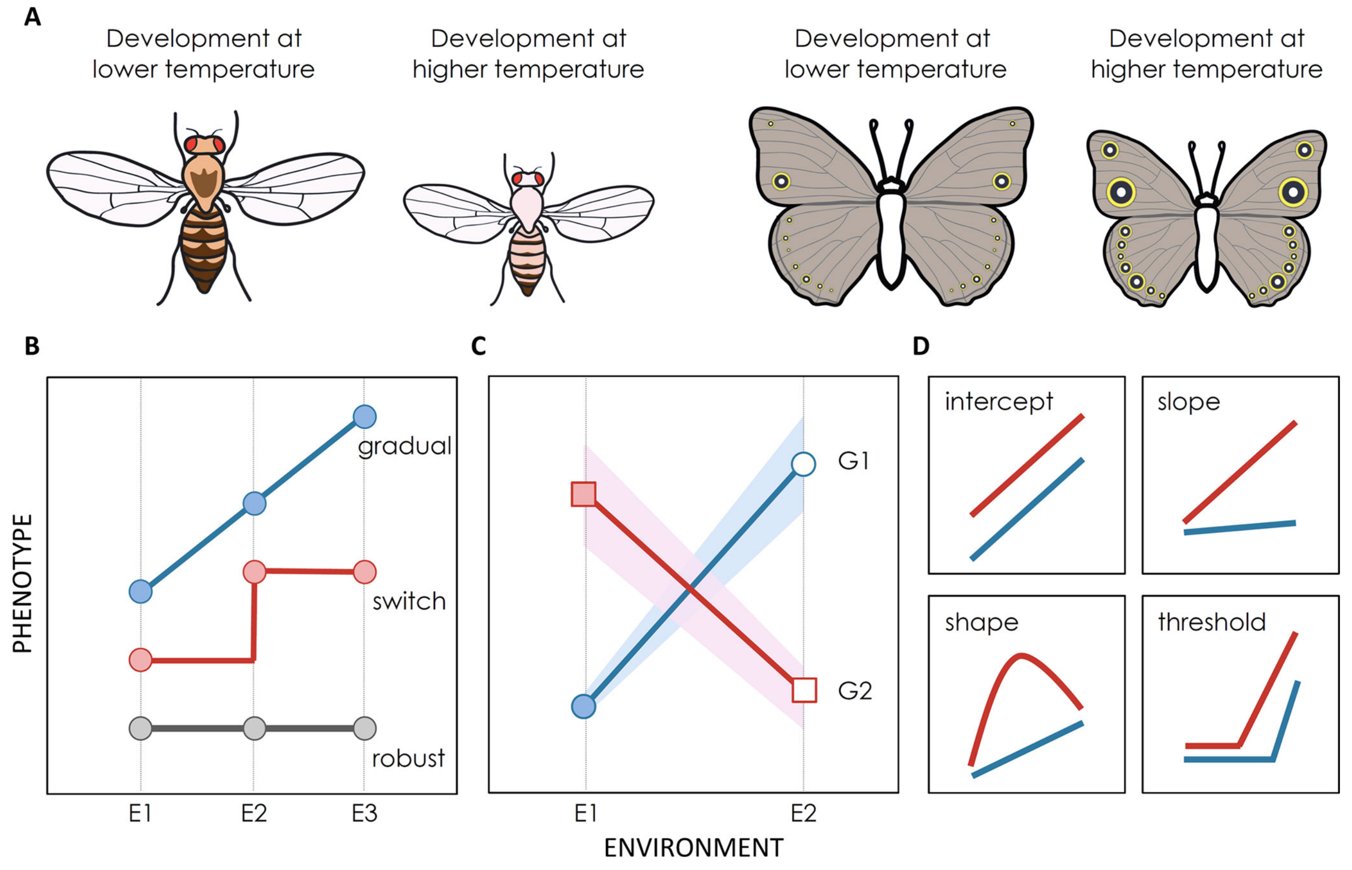
- The holobiont is a single dynamic entity that a vast amount of the genetic information and variability are contributed by the microorganisms
    - The genetic information encoded by microorganisms can change under environmental requirements more rapidly, and by more processes, than the genetic information encoded by the host organism

The genetic information encoded by a microbial symbiont population can change in 3 ways

1. Alterations in the relative abundances of microorganisms
2. Through introduction of new microorganisms from the environment
3. Genetic alteration of existing microbes through mutation, horizontal gene transfer, and selection of those changes

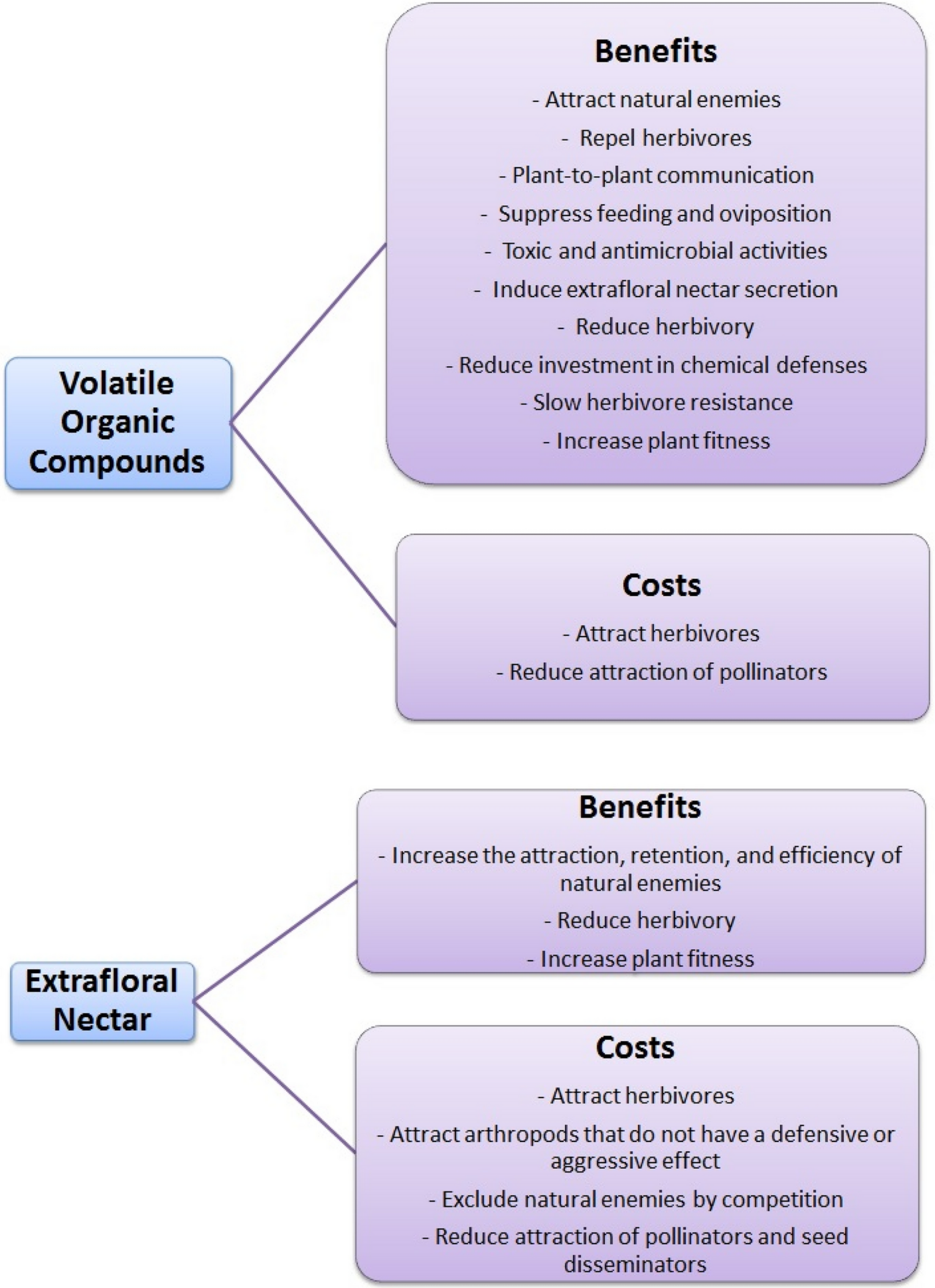
- Developing organisms alter their morphology, physiology, and behaviours in response to environmental conditions

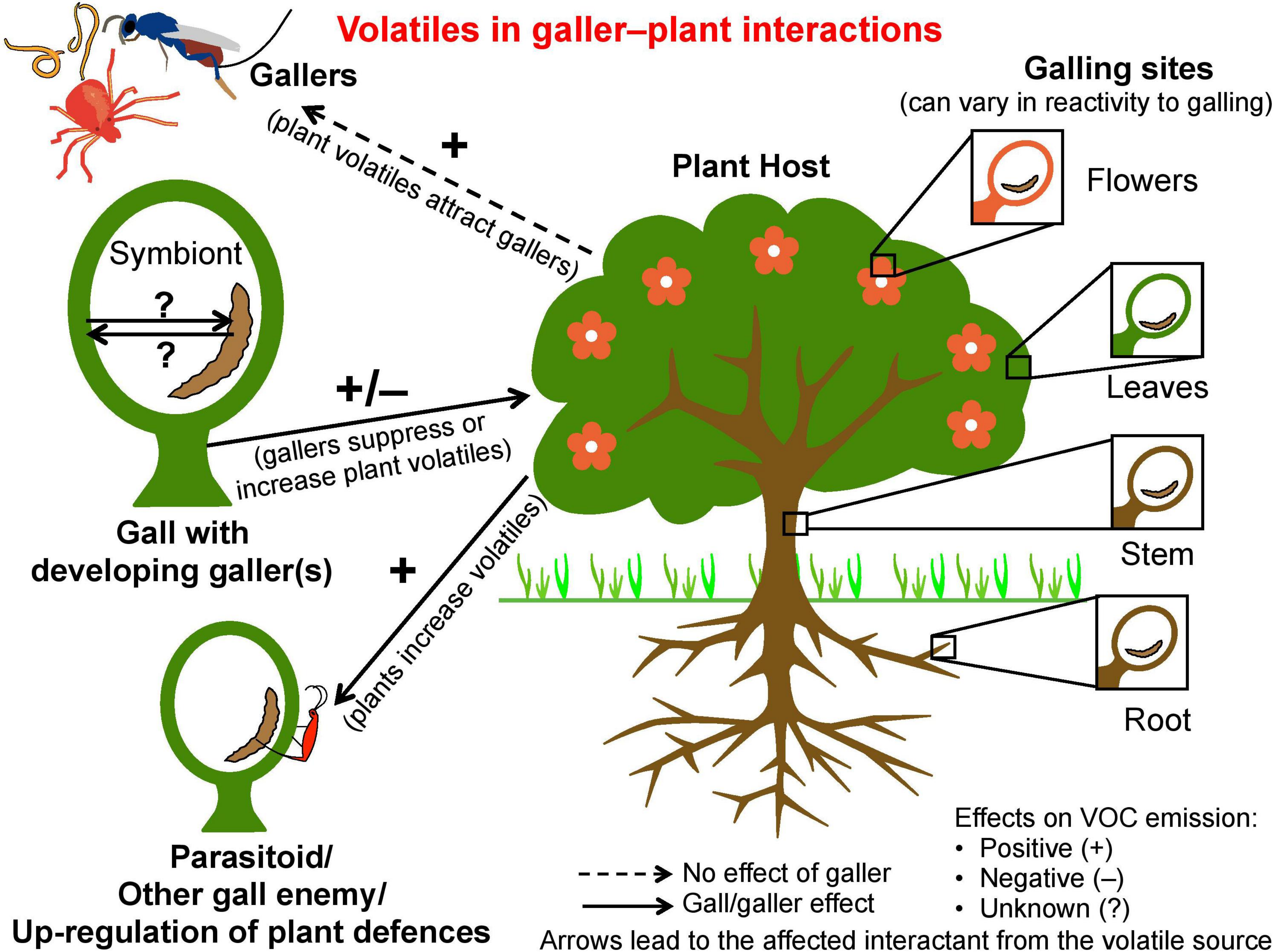
- The environment is not only a selective filter, developmental plasticity can transform the environment into a an active agent shaping the phenotype



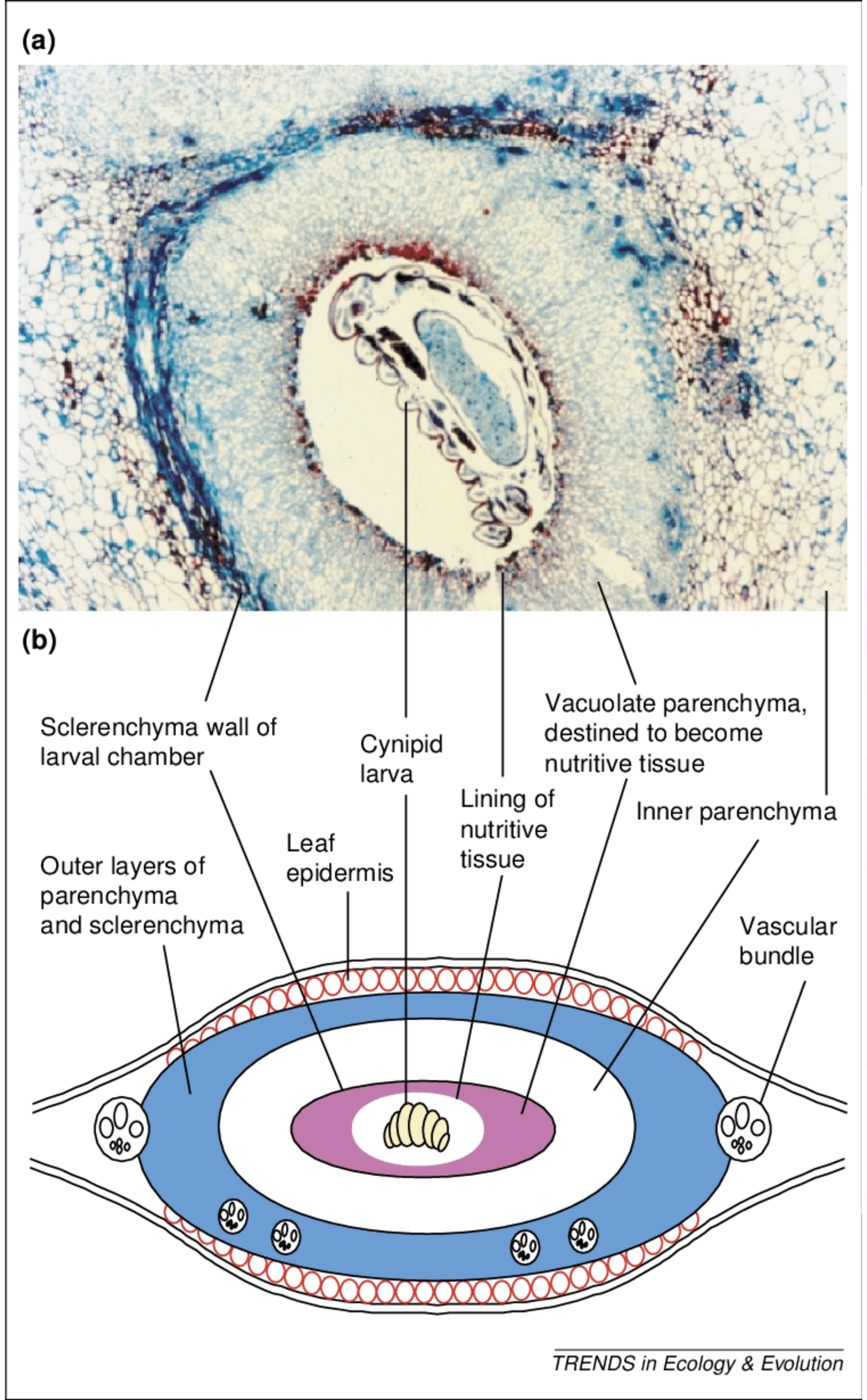
- Two large sources of indirect herbivory defense are volatile organic compounds and extrafloral nectar

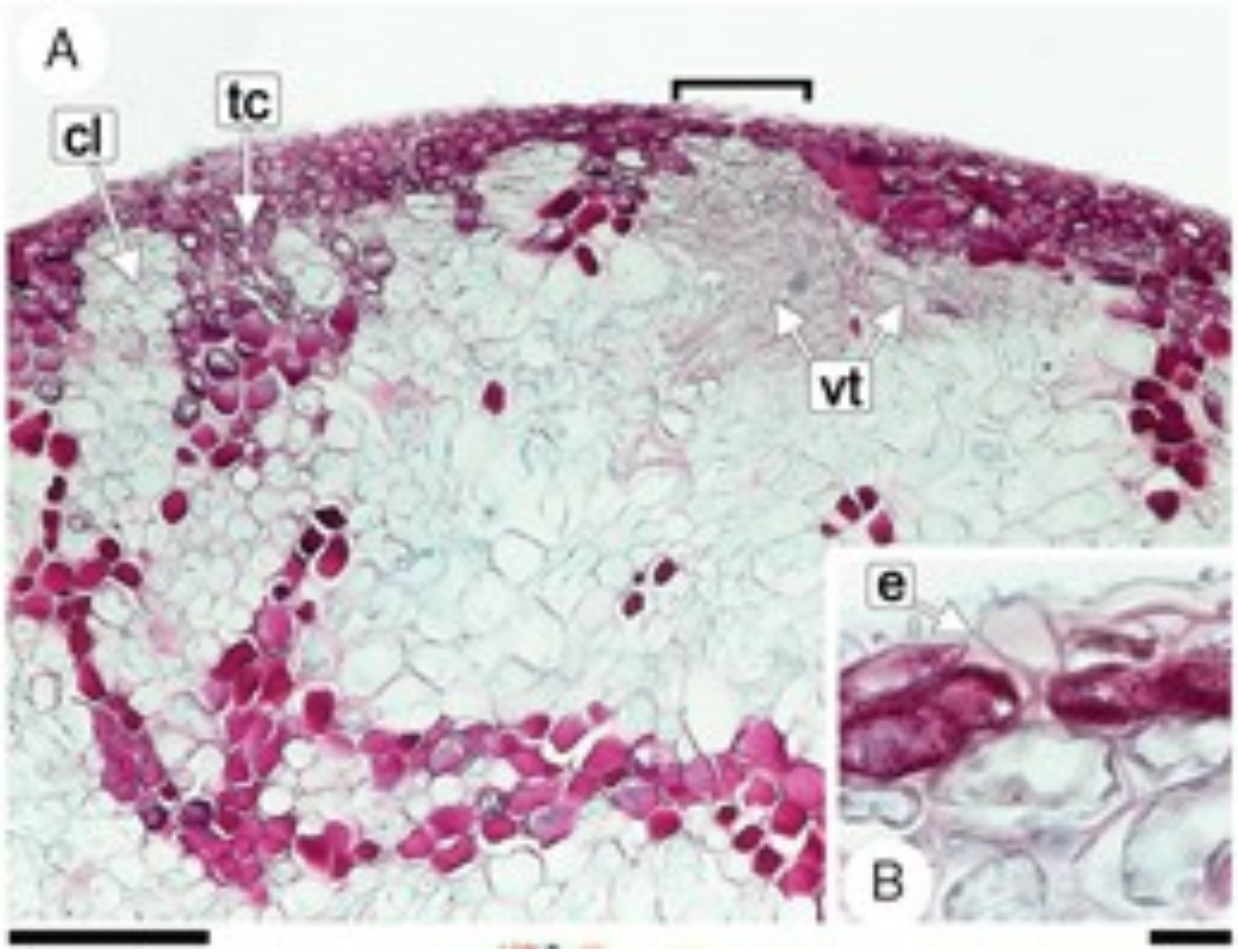
- Both traits have been shown to be very plastic in plants, and can change in response to a large variety of factors

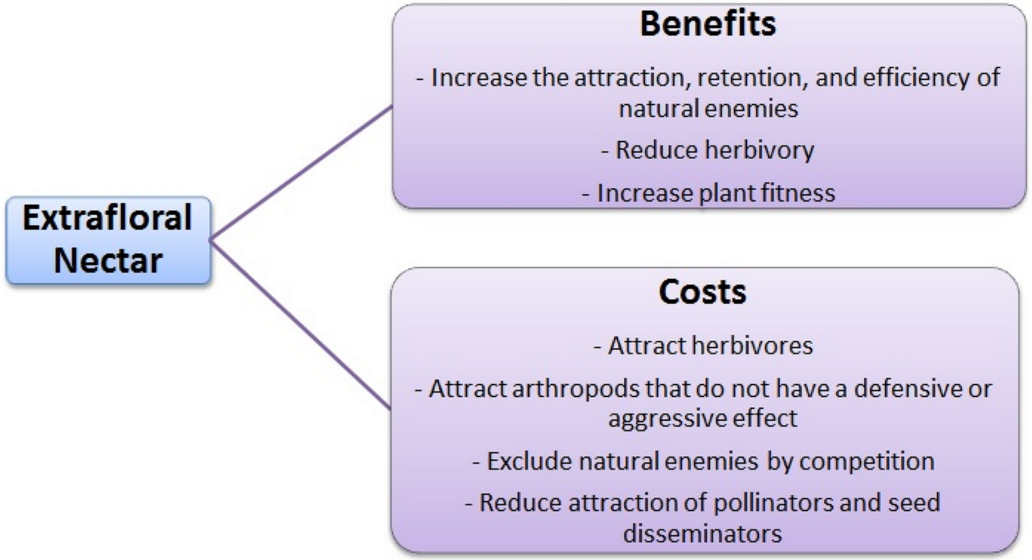




Volatiles in galler–plant interactions
Gallers
+
(plant volatiles attract gallers)
Galling sites
(can vary in reactivity to galling)
Plant Host
Flowers
Leaves
Stem
Root
Symbiont
?
?
+/–
(gallers suppress or increase plant volatiles)
Gall with developing galler(s)
+
(plants increase volatiles)
Parasitoid/
Other gall enemy/
Up-regulation of plant defences
No effect of galler
Gall/galler effect
Effects on VOC emission:
• Positive (+)
• Negative (–)
• Unknown (?)
Arrows lead to the affected interactant from the volatile source


(a)
(b)
Sclerenchyma wall of larval chamber
Cynipid larva
Lining of nutritive tissue
Vacuolate parenchyma, destined to become nutritive tissue
Inner parenchyma
Outer layers of parenchyma and sclerenchyma
Leaf epidermis
Vascular bundle
TRENDS in Ecology & Evolution


A
cl
tc
vt
e
B



- Cynipid galls that release nectar act in a similar fashion to EFNs
- Oaks almost ubiquitously have no EFNs, outsourcing this trait to symbiont cynipids

Discussion

Where is the line between environment and evolutionary unit

Are signals from the environment vs from a symbiont going to act differently?

Is controlling that microbiome a form of selection on the holobiont or plastic responses of the holobiont?

Is it worth thinking in terms of the holobiont or hologenome?

A lot of the developmental or lethal defects that come from altered microbiomes are often rescued when inoculated with the proper microbes

Most people focus on mutualisms, should parasites be considered a part of the holobiont?

What constitutes a signal from or a response to the environment compared to a symbiont?