

# *Comparative genomics of cellulolytic shipworm symbionts*



**PGC Webinar**  
**August 9, 2016**

Philippine Mollusk Symbiont – International Cooperative Biodiversity Group  
Marvin A. Altamia

# About our research project:

The scope of the **Philippine Mollusk Symbiont International Cooperative Biodiversity Group (PMS-ICBG)** includes biodiversity, drug discovery, biofuels and basic research. The focus is on bacteria associated with mollusks in one of the world's hotspots of diversity, the Philippines.

## **Principal Investigator:**

Margo Haygood (University of Utah)

## **Co-Investigators:**

Daniel Distel (Ocean Genome Legacy, Northeastern University)

Gisela Concepcion (Marine Science Institute, University of the Philippines)

Hiroaki Naka (Oregon Health & Science University)

Eric Schmidt (University of Utah)

Baldomero Olivera (University of Utah)

Gary Rosenberg (Drexel University)

**Website:** [www.pmsicbg.org](http://www.pmsicbg.org)

**Funding agency:** US-NIH

# Shipworms are wood-boring marine bivalves related to clams



Shipworm burrow in mangrove wood



Wood panels can be used as shipworm baits

# The valves (shells) that protect the body of clams evolved and became a drill bit in shipworms



mouth

Microscopic rasp-like projections in the valves allow the shipworm to burrow and reduce the wood in to tiny ingestible particles.

# Shipworms are also known as 'termites of the sea'



Pine board infested with shipworms. From Daniel Distel's laboratory  
at Ocean Genome Legacy, Northeastern University

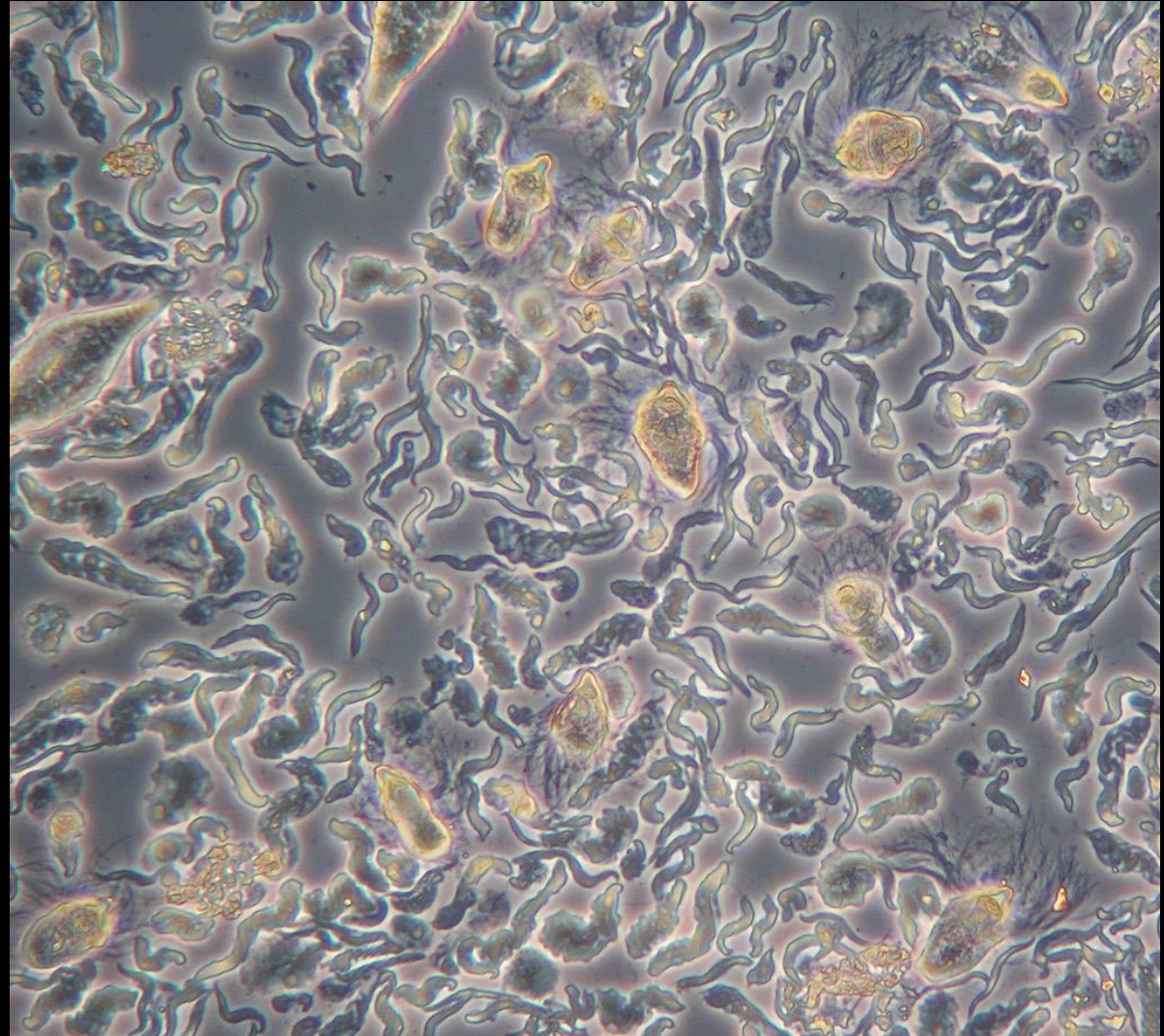
# Almost all animals that feed on wood (lignocellulose) have symbionts



- in almost all cases, the symbionts that degrade lignocellulose reside in the gut

# Many xylophagous animals have a complex microbiota in their digestive tract

- photomicrograph of termite hindgut
- bacteria, archaea, protozoa, and fungi are in direct contact with the ingested wood particles
- VERY HIGH COMPLEXITY = HEADACHE



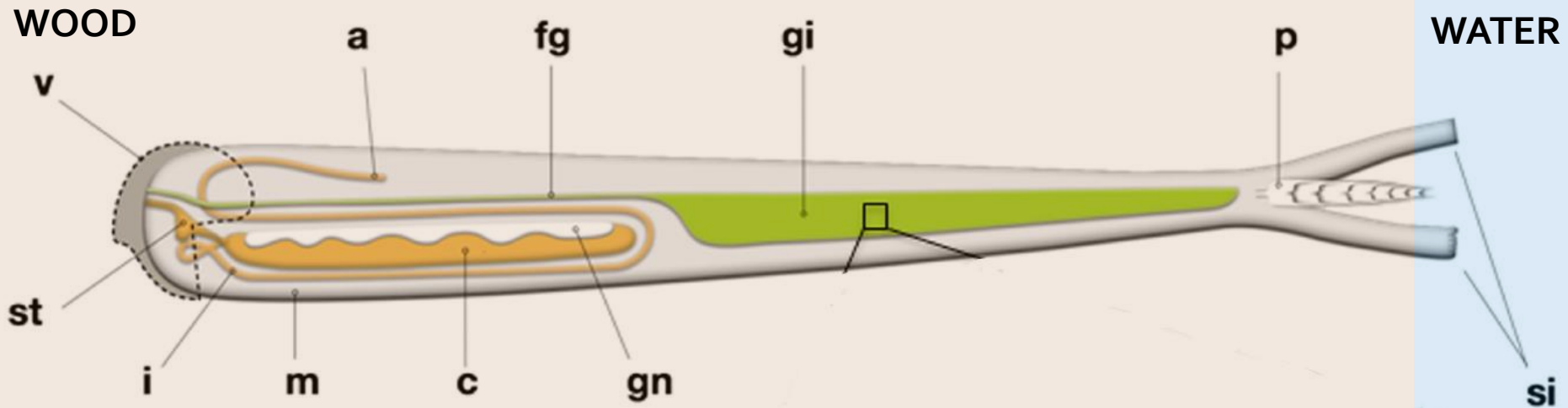
Shipworms can be maintained in the lab



saltwater aquaria at Dan Distel's Lab at Ocean Genome Legacy



# The two feeding modes of shipworms



## Pathway for ingested wood

- v valves
- m mouth
- st stomach
- c cecum
- i intestine
- a anus

## Pathway during filter-feeding

- si siphon
- gi gills
- fg food groove

## Other parts

- p pallets
- gn gonads

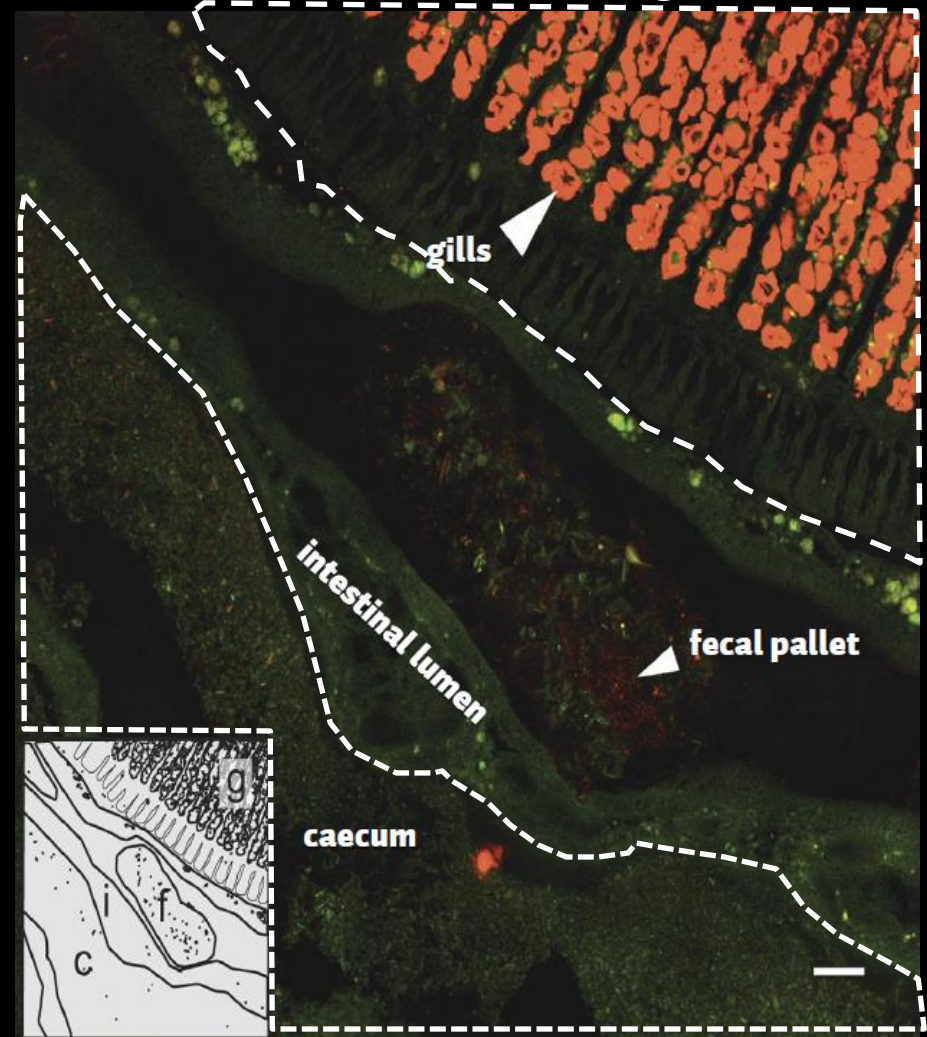
ciliary motion

**Gills and digestive tract are physically connected**

# What's weird about shipworms?

- cecum, the primary wood-digesting organ, has very few microbes
- carbohydrate-active enzymes (CAZymes) can be detected in the caecum
- **gills contain bacterial endosymbionts**

Fluorescence *in situ* hybridization (FISH) to detect bacteria in organs

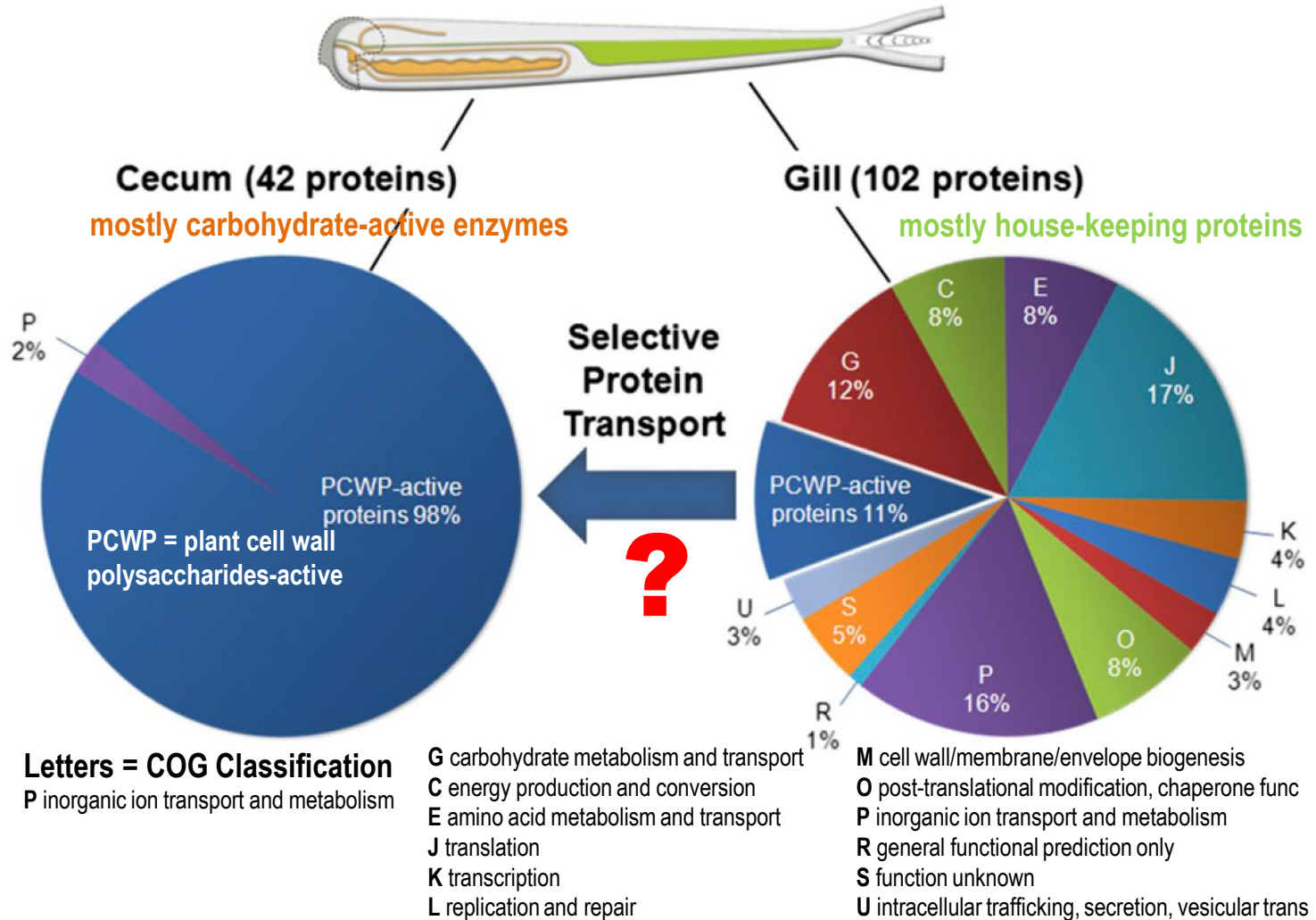


**Red signals = bacteria**

# Gill bacteria-derived enzymes can be detected in the cecum using proteomics

## PROTOCOL:

1. Proteins from gills and cecum contents were extracted by boiling
2. RP-HPLC fractionation
3. Tryptic digestion, then tandem MS
4. Peptide sequences were searched against *in silico* digested gill metagenome and isolate genomes



# *Teredinibacter turnerae* is the main bacterial partner of many shipworms

- **intracellular:** found inside specialized cells in the gills of shipworms
- **cultivable** but so far has never been detected in the environment
- Gram-negative ( $\gamma$ -proteobacteria, Family Alteromonadaceae)
- grows on seawater-based cellulose medium and can fix atmospheric dinitrogen (John Waterbury's 1983 Science Magazine paper)
- closed circular genome: 5.19 Mbp
- genome codes for high number of carbohydrate-active enzymes (CAZymes) that target the components of wood (lignocellulose)—  
**potential applications in biofuel production**
- numerous secondary metabolite gene clusters, hence, might be useful for **antimicrobial drug discovery**

# *T. turnerae* can digest naturally occurring substrates *in vitro*



negative control

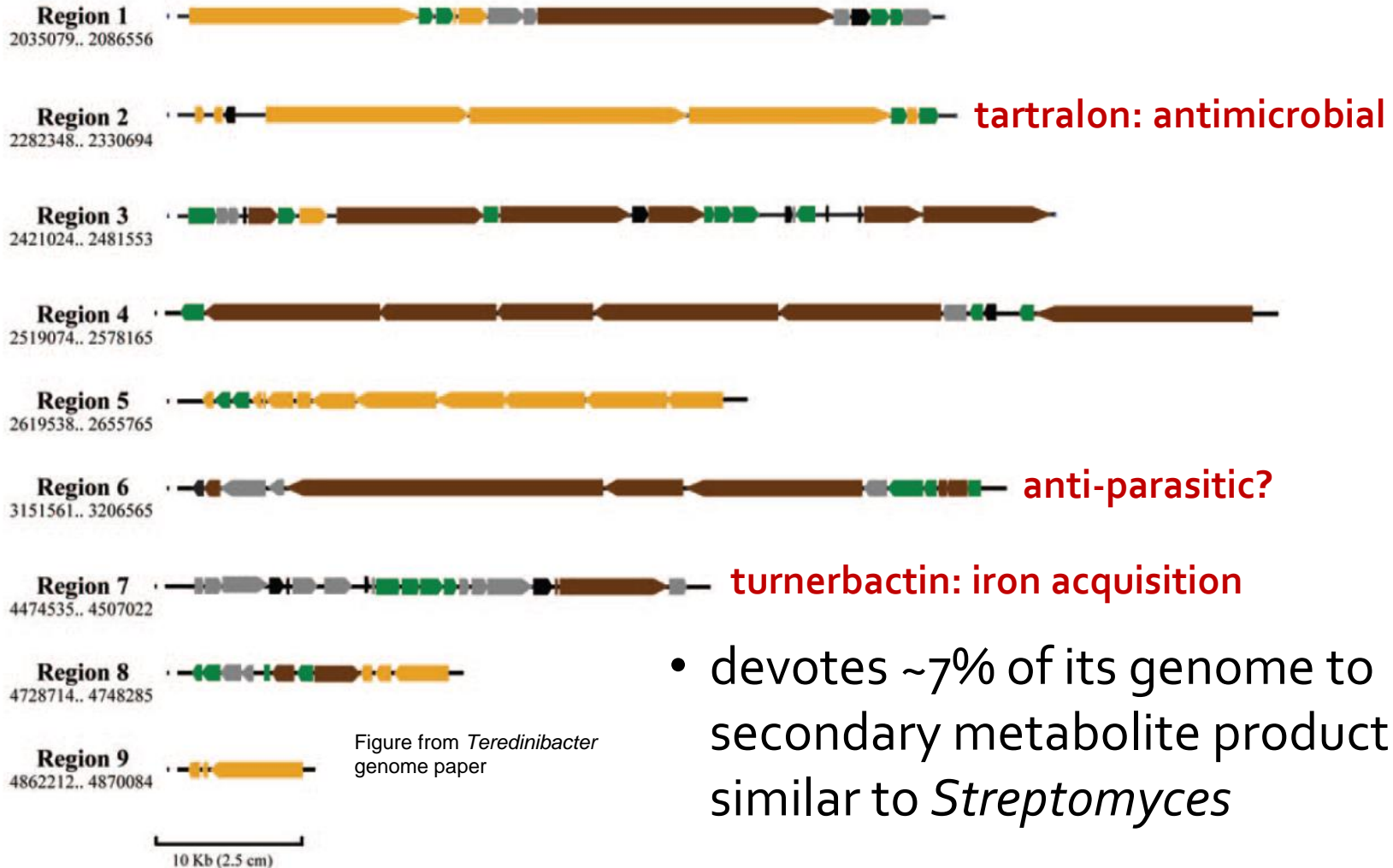


inoculated with *T. turnerae*

substrates were placed on a seawater-based broth medium with  $\text{NH}_4\text{Cl}$   
and were then sterilized using an autoclave

**POTENTIAL APPLICATIONS FOR BIOFUEL PRODUCTION**

# *T. turnerae* has numerous secondary metabolite gene clusters



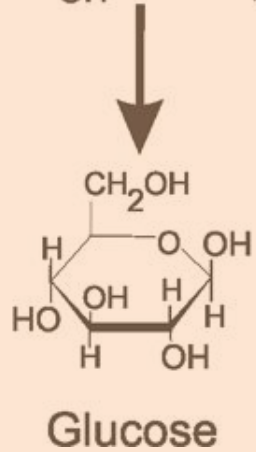
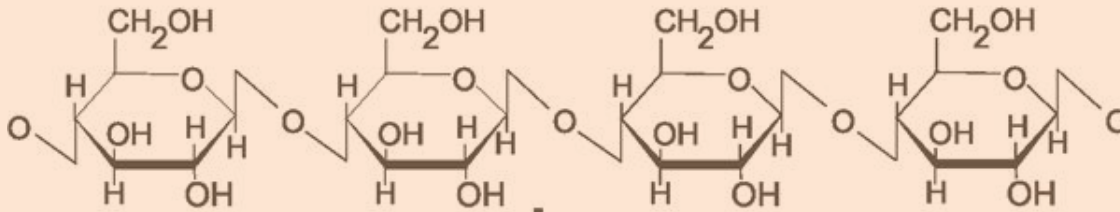
- devotes ~7% of its genome to secondary metabolite production similar to *Streptomyces*

POTENTIAL APPLICATIONS FOR DRUG DISCOVERY

# PMS-ICBG's hypothesis aka ecorationale

## SHIPWORM CECUM

Cellulose



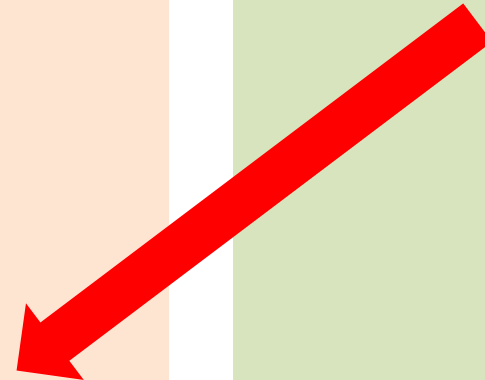
cellulases and  
other enzymes

opportunistic  
bacteria love  
simple sugars

ANTIMICROBIAL  
COMPOUNDS

## SHIPWORM GILLS

endosymbiotic  
bacteria



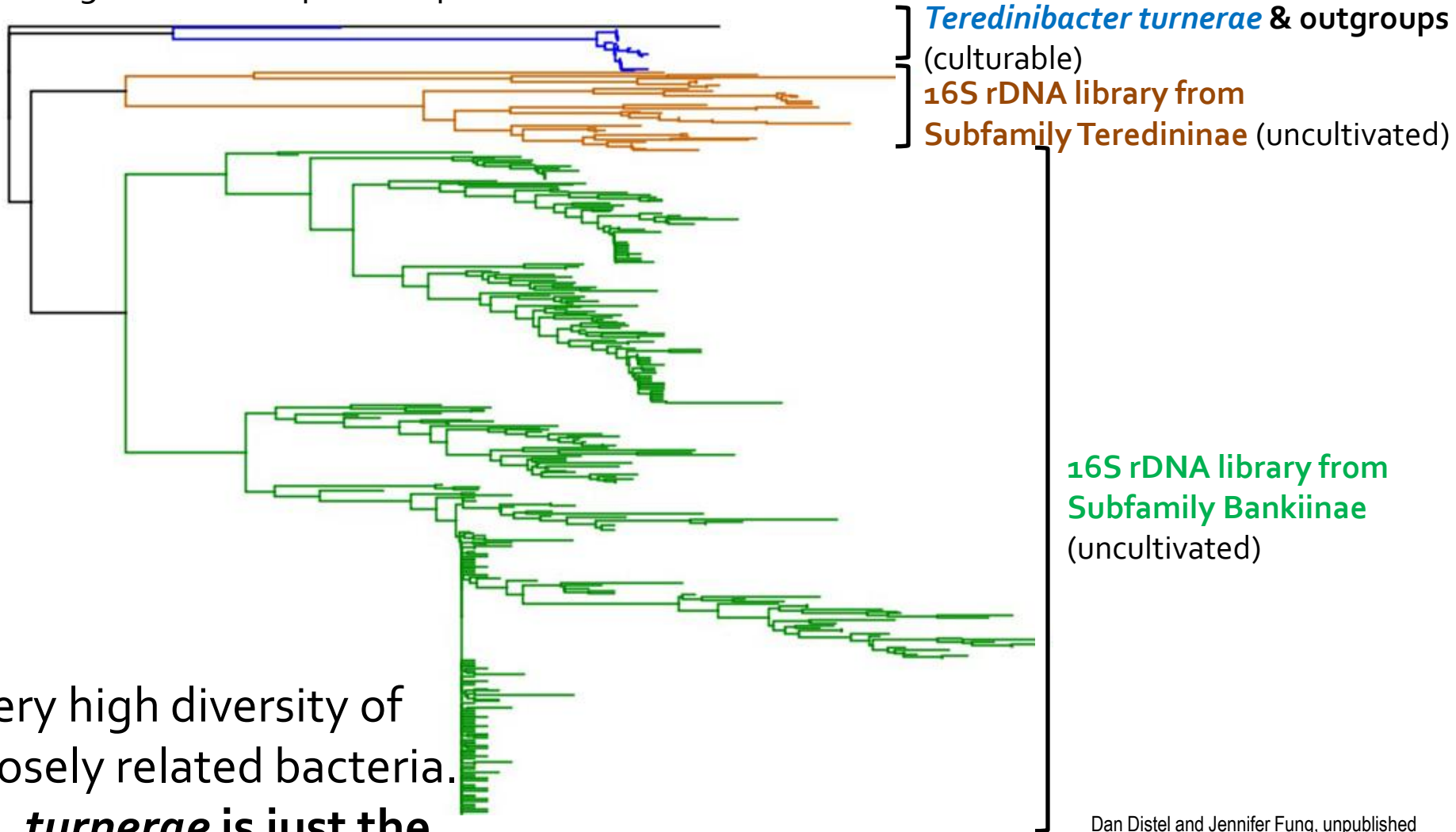
Can we cultivate shipworm  
symbionts other than  
*Teredinibacter turnerae*?





# *T. turnerae* is not the only shipworm symbiont

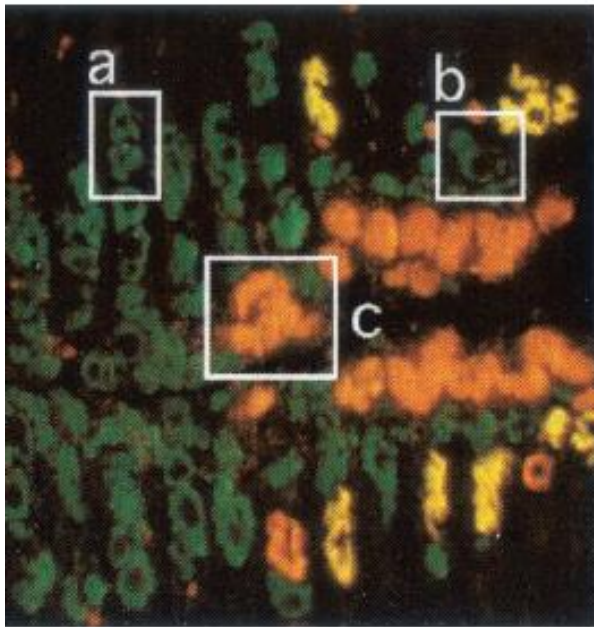
Phylogenetic analysis of 16S rDNA clone libraries prepared from gills of two shipworm species.



Dan Distel and Jennifer Fung, unpublished

Very high diversity of  
closely related bacteria.  
*T. turnerae* is just the  
tip of the iceberg.

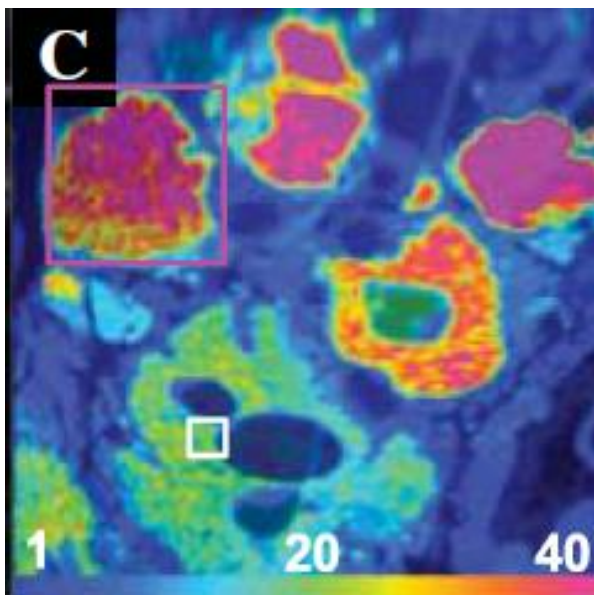
# *T. turnerae* is not the only shipworm symbiont



Some bacteriocytes in the gill of *Lyrodus pedicellatus* do not hybridize with *Teredinibacter turnerae*-specific FISH probe... **segregation of symbionts**

Distel, et. al, AEM, vol. 68, p. 6292, 2002

- *T. turnerae*-specific probe
- eubacterial probe
- colocalization



Variation in the level of N<sub>2</sub>-fixation in shipworm gill bacteriocytes...

Lechene, et. al,  
Science, vol. 317, p. 1563, 2007



**Most of the shipworms we  
collect come from mangrove areas**

A group of people are on a boat, examining large pieces of mangrove wood. The wood is heavily infested with shipworms, which are visible as small, white, worm-like creatures. The boat is yellow and green, and the background shows a body of water and distant mountains under a cloudy sky. The text "Mangrove wood infested with shipworms" is overlaid on the image.

**Mangrove wood infested  
with shipworms**

Shipworms are coaxed out of the wood using carpentry tools



# Giant shipworm tunnel inside a mangrove branch



Shipworms line their tunnels  
with calcareous material

Shipworm extracted  
out of the wood are  
then photographed

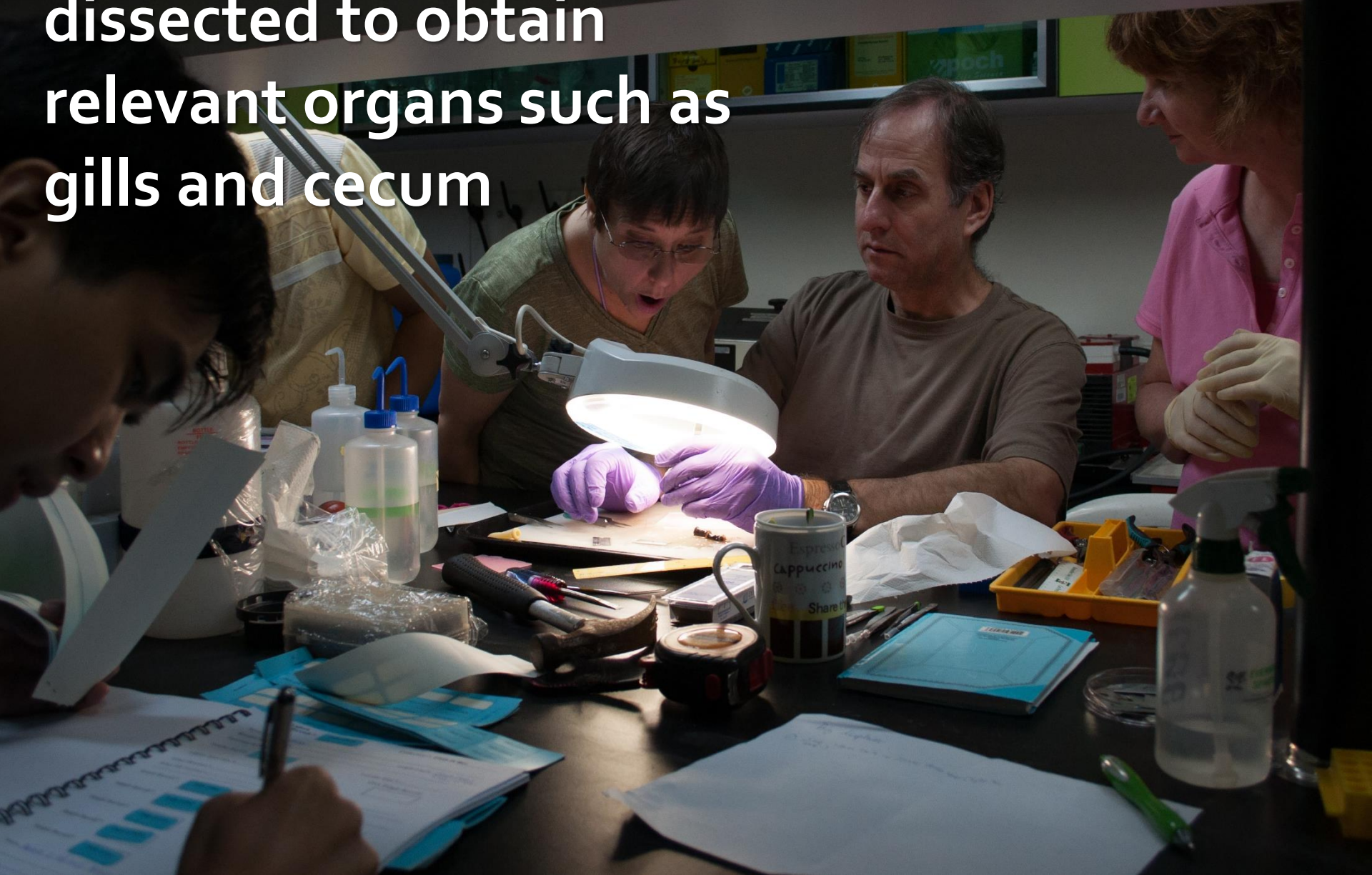


**Some shipworm species  
collected here in the Philippines**

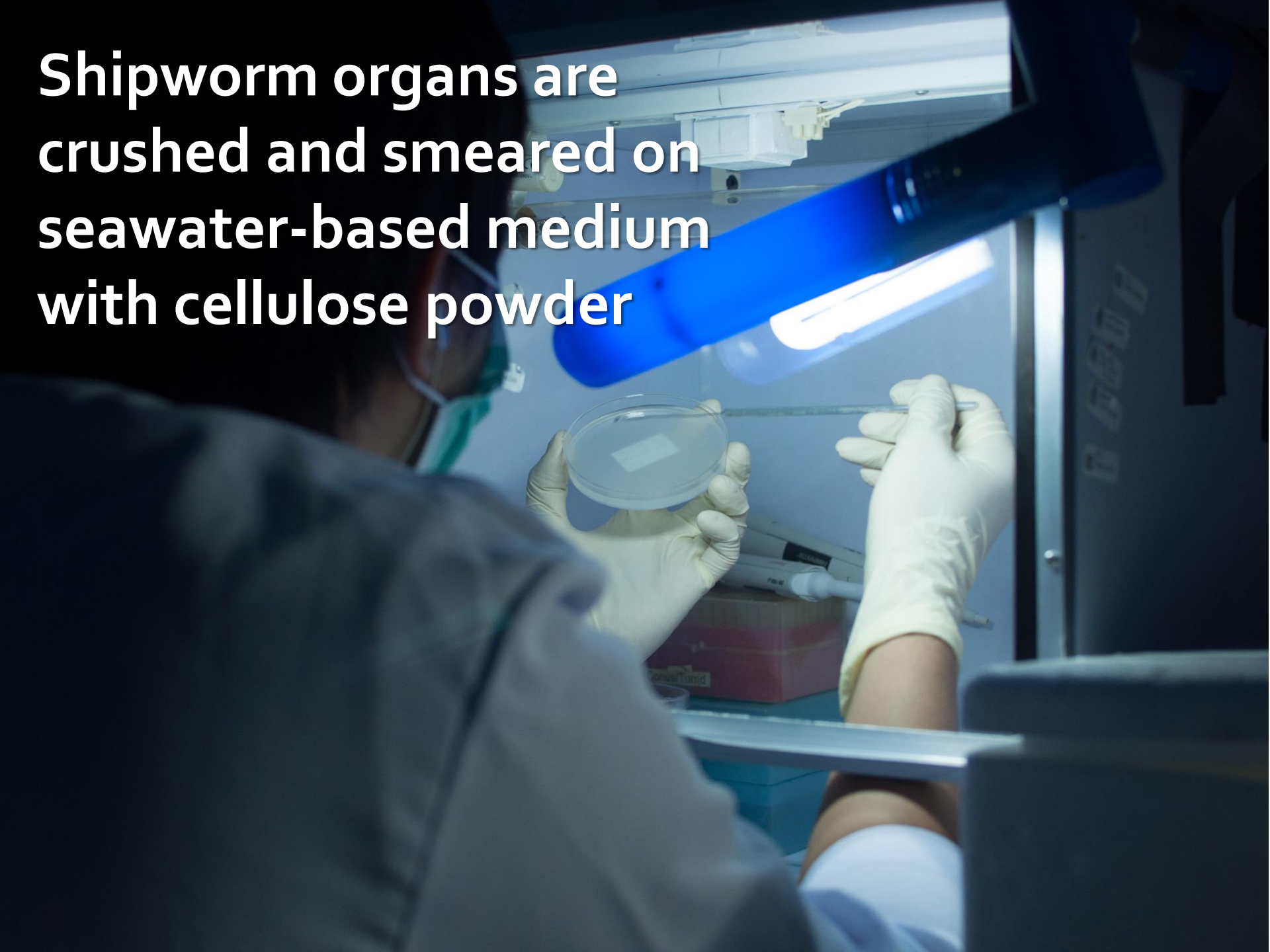
**REDACTED**



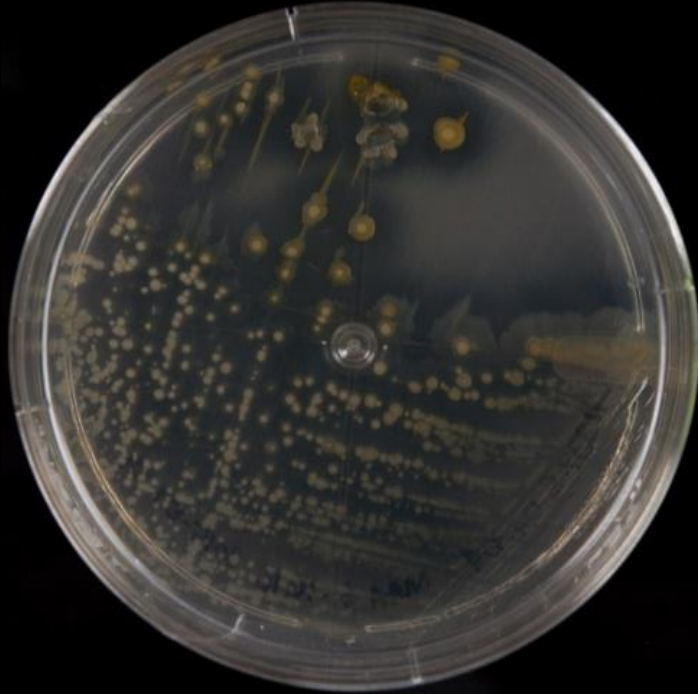
Shipworms are then dissected to obtain relevant organs such as gills and cecum



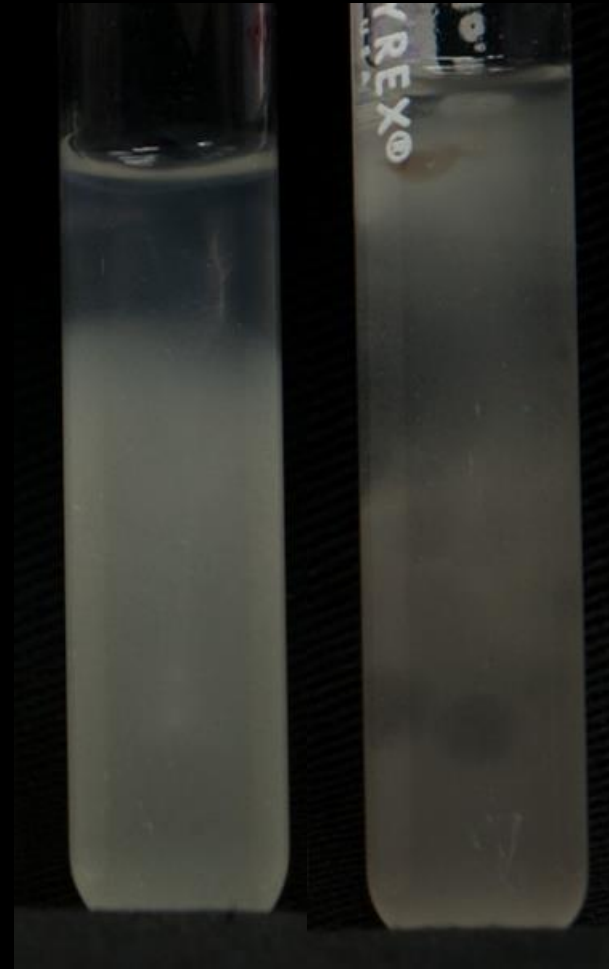
**Shipworm organs are  
crushed and smeared on  
seawater-based medium  
with cellulose powder**



# Cellulose-degrading bacteria isolated from shipworms



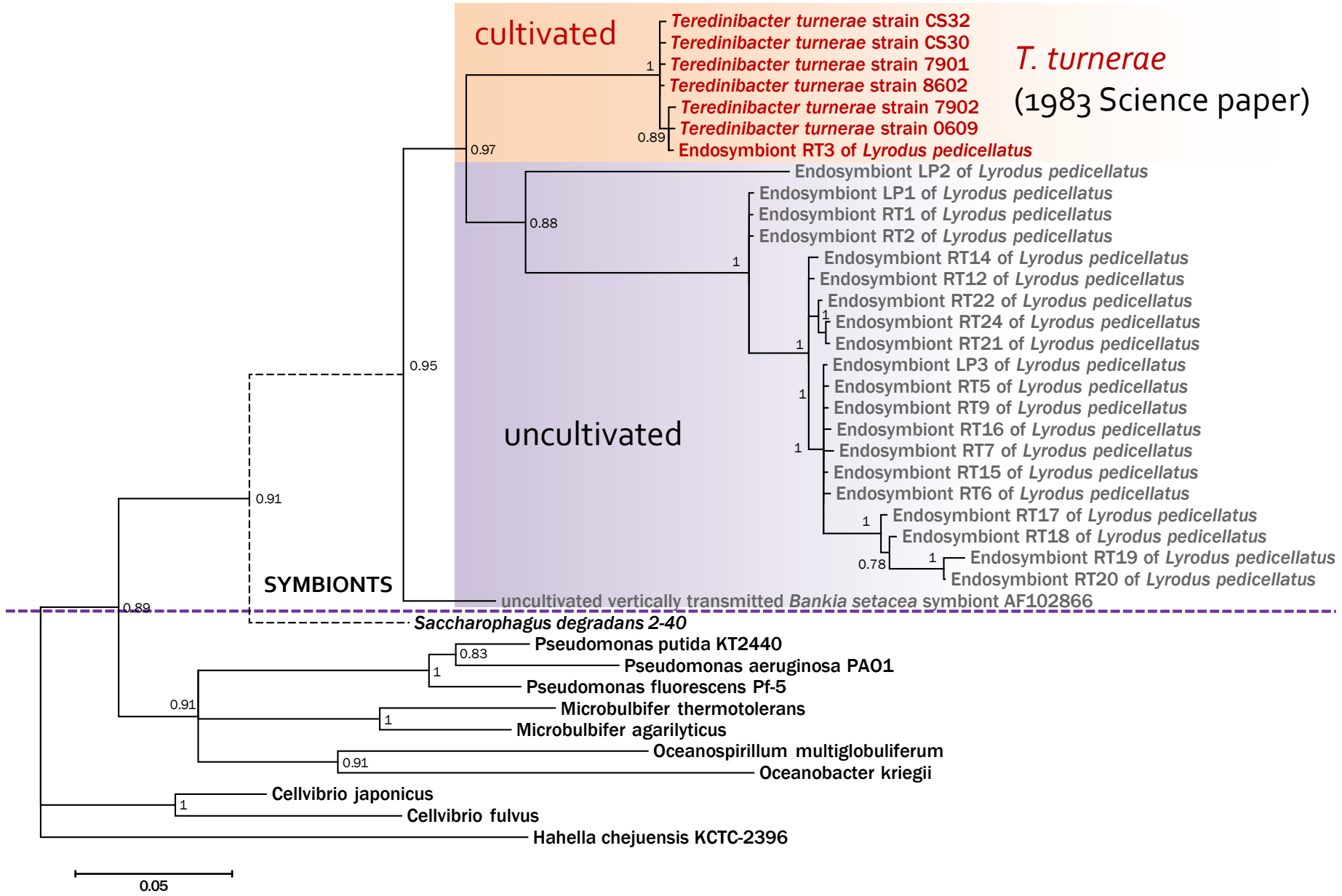
Cellulolytic bacteria produce halos/clearings in media containing powdered cellulose.



# Some novel shipworm cellulolytic and agarolytic isolates

**REDACTED**

# 16S rDNA phylogenetic tree before PMS-ICBG



# 16S rDNA phylogenetic tree during PMS-ICBG

Bayesian tree constructed using  
1,233 bp nucleotide positions

★ novel isolates

Gammaproteobacteria under  
Family Alteromonadaceae

10 novel isolates:

- 9 cellulolytic
- 1 agarolytic

3 additional *T. turnerae* stains  
from the Philippine shipworms

**REDACTED**

*Saccharophagus degradans*  
is the closest free living relative

# Whole genome sequencing of the isolates

1. One liter broth culture.
2. Genomic DNA was isolate using Joint Genome Institute's CTAB-phenol chloroform extraction protocol developed for *Chlamydomonas*.
3. gDNA samples QC'ed using NanoDrop and standard gel electrophoresis. Concentration was measured densitometrically.
4. Joint Genome Institute-Department of Energy sequenced our isolates **FOR FREE!**
5. JGI used Illumina and/or PacBio platforms to generate "Improved Microbial Draft" sequences.
6. Sequence QC, assembly, and annotation all done in-house at JGI.

**Processing time:** 168-491 days (average of 282 days or 9.2 months)

# Genomes of cellulolytic/agarolytic shipworm symbionts and isolates

**REDACTED**



# Genome size comparison

**REDACTED**

# Genomes of shipworm symbionts vs other intracellular symbionts

**REDACTED**

**Dinitrogen fixers, anaerobic nitrate respirers,  
and xenobiotic detoxifiers**

**REDACTED**

# Carbohydrate-active enzymes (CAZymes)

- **modular enzymes**, each module can catalyze the breakdown, biosynthesis or modification of carbohydrates and glycoconjugates

## Enzymatic modules:

1. Glycoside Hydrolases (GH): hydrolysis and/or rearrangement
2. Glycosyltransferases (GT): formation of glycosidic bonds
3. Polysaccharide Lyases (PL): hydrolytic cleavage
4. Carbohydrate Esterases (CE): hydrolysis of carbohydrate esters
5. Auxiliary Activities (AA): redox enzymes

Modules can be further classified into families based on their protein structure and function

## Non-catalytic modules:

1. Carbohydrate-binding Modules (CBM): adhesion to carbohydrates

## Example of a CAZyme:



secretion signal (may or may not be present)

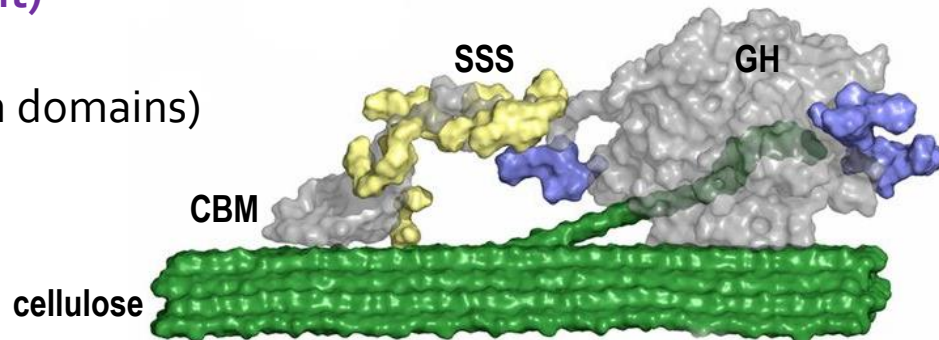
CE domain

SSS (polylinker, provides flexibility between domains)

CBM domains

GH domain

*Trichoderma reesei* CAZyme



# Identification and classification of CAZymes from shipworm symbiont genomes

- BLAST doesn't work because of multiple domains
- *Bankia setacea* isolates were processed by the group Bernard Henrissat (creator of CAZy Database)
- for Philippine isolates, we used dbCAN (HMM-based prediction) (<http://csbl.bmb.uga.edu/dbCAN/index.php>)
- manually rechecked the dbCAN's output

Query	Subject	E-value	Start	End	Covered fraction	Start	End
P886DRAFT_1937	<a href="#">GH11</a>	8.30E-64	2	176	0.983	53	235
	<a href="#">CBM60</a>	5.00E-42	2	107	0.972	264	368
	<a href="#">CE6</a>	3.00E-26	1	99	0.990	479	588
	<a href="#">CBM57</a>	4.00E-39	1	145	0.980	691	826
	<a href="#">CBM5</a>	4.90E-08	4	40	0.900	908	945
	<a href="#">CBM57</a>	6.70E-36	1	144	0.973	997	1131
	<a href="#">CE15</a>	2.50E-89	2	268	0.989	1180	1502
P886DRAFT_2039	<a href="#">CBM50</a>	0.00085	1	27	0.650	177	211

- SignalP to determine if a secretion signal is present

# Carbohydrate binding & catalytic domains

**REDACTED**

# Enzymatic degradation of common components of woody plant materials

**REDACTED**

# Multidomain / multifunctional predicted CAZymes from shipworm symbionts/isolates

**REDACTED**



# Substrate specificity of GH and PL modules

**REDACTED**

**Cellulolytic shipworm symbionts have more GH modules specific for plant polysaccharides compared to generalists**

**REDACTED**

# **GH modules that target other polysaccharides**

**REDACTED**

**Shipworm symbiont CBMs show specificity towards cellulose/xylan**

**REDACTED**

# CAZymes from shipworm bacteria

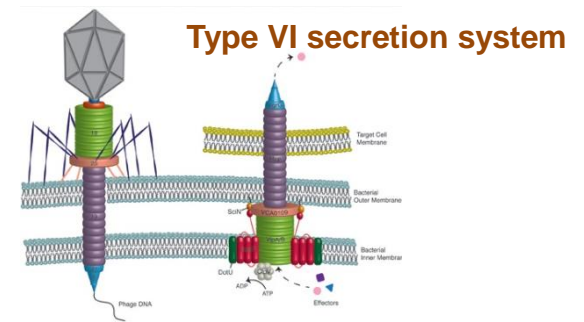
**REDACTED**

**Quorum sensing, phage defense systems,  
restriction-modification systems, CRISPRs**

**REDACTED**

# Secretion systems

REDACTED



# Are secondary metabolite gene clusters present in other shipworm symbiont genomes?

- *T. turnerae* devotes a huge percentage of its genome to secondary metabolite production
- are shipworm symbionts defensive symbionts?
- biosynthesis clusters can be predicted using online tools (e.g. antiSMASH, IMG/ABC)

## Some classes of biosynthetic clusters that can be predicted:

**NRPS:** nonribosomal peptide synthases (e.g. vancomycin, actinomycin)

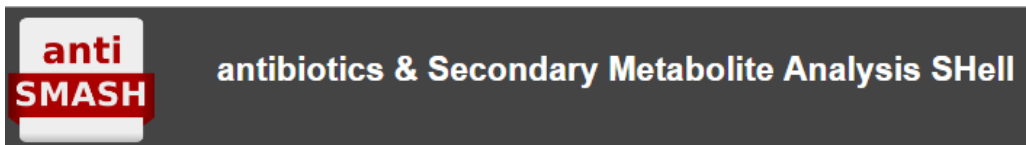
**PKS:** polyketide synthases (e.g. doxorubicin, erythromycin)

**hybrid NRPS/PKS:** (e.g. bleomycin)

**quorum signaling molecules:** (e.g.  $\gamma$ -butyrolactones, homoserine lactones, etc.)

**$\beta$ -lactams:** penicillin

**bacteriocins:** ribosomally synthesized antimicrobial peptides (e.g. nisin)

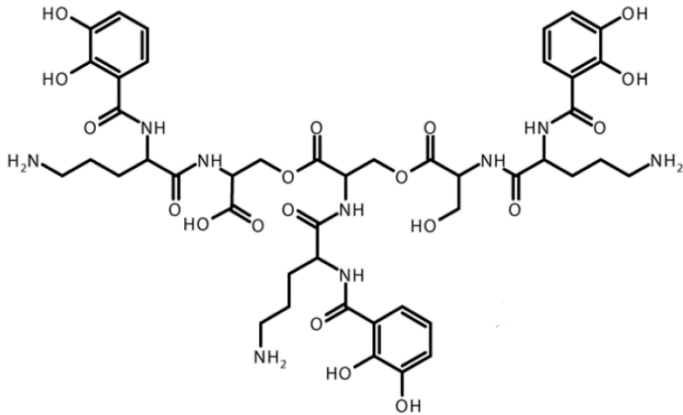


<https://antismash.secondarymetabolites.org>



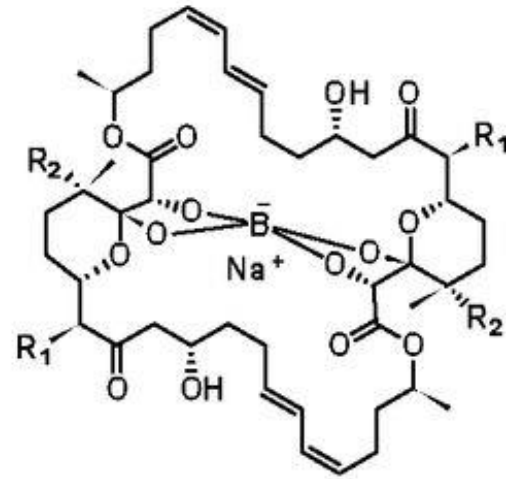
# Characterized secondary metabolites of *Teredinibacter turnerae*

- turnerbactin (*tnb*), a triscatecholate siderophore, is produced by *tnb* NRPS



- present in all *T. turnerae* genomes examined
- identical clusters found in novel isolates Bso8 and PMS-1120W

- boronated tartrolon product of a PKS cluster



- antimicrobial
- can be detected on whole shipworm organic extracts

**There is competition among  
shipworm symbionts**

**REDACTED**

# Secondary metabolite gene clusters of shipworm symbionts and isolates

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# Related secondary metabolite gene clusters

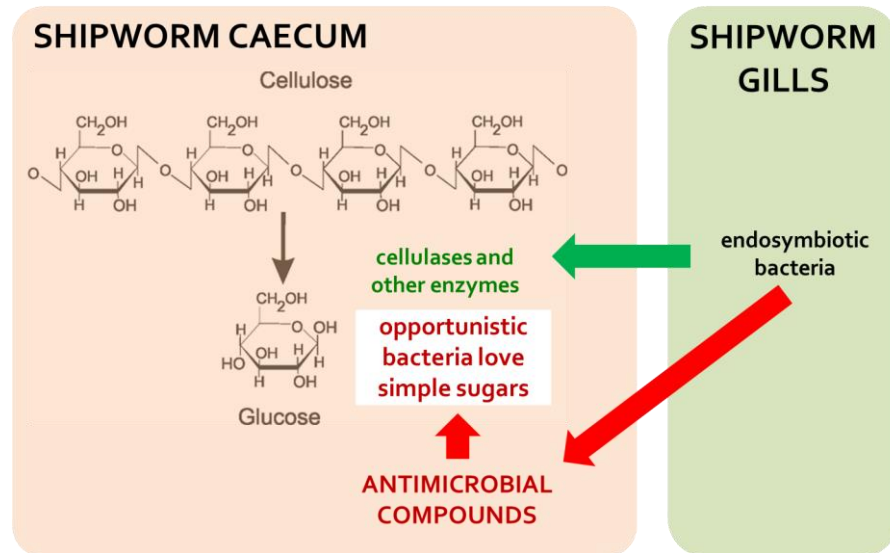
**REDACTED**

# Emerging patterns in shipworm symbionts/isolates:

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# SUMMARY:

- shipworm symbionts have not yet undergone genome reduction observed in obligate intracellular symbionts
- potential division of labor
- rich sources of CAZymes that target plant polysaccharides
- presence of numerous secondary metabolite clusters suggests that are also defensive symbionts
- our project's hypothesis is supported by the shipworm symbiont genome data



# Acknowledgements (past and present members)

- **Joint Genome Institute-Department of Energy for performing whole genome sequencing**
- UP-MSI (Irene, Jose Miguel, Jortan, Lilibeth, Malem, MERF, Melfeb, MJ, Noel, Clarisse, Gwen, Imelda, Mary Anne)
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- Caraga State U (Romell Seronay)
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  - Kalamansig, Sultan Kudarat

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*thanks for listening...*

