



NGS para el estudio de la diversidad microbiana

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Investigador Titular C



**UNU
BIOLAC**



Curso internacional

Secuenciación y análisis de datos genómicos para la detección microbiológica de enfermedades transmitidas por alimentos y aguas



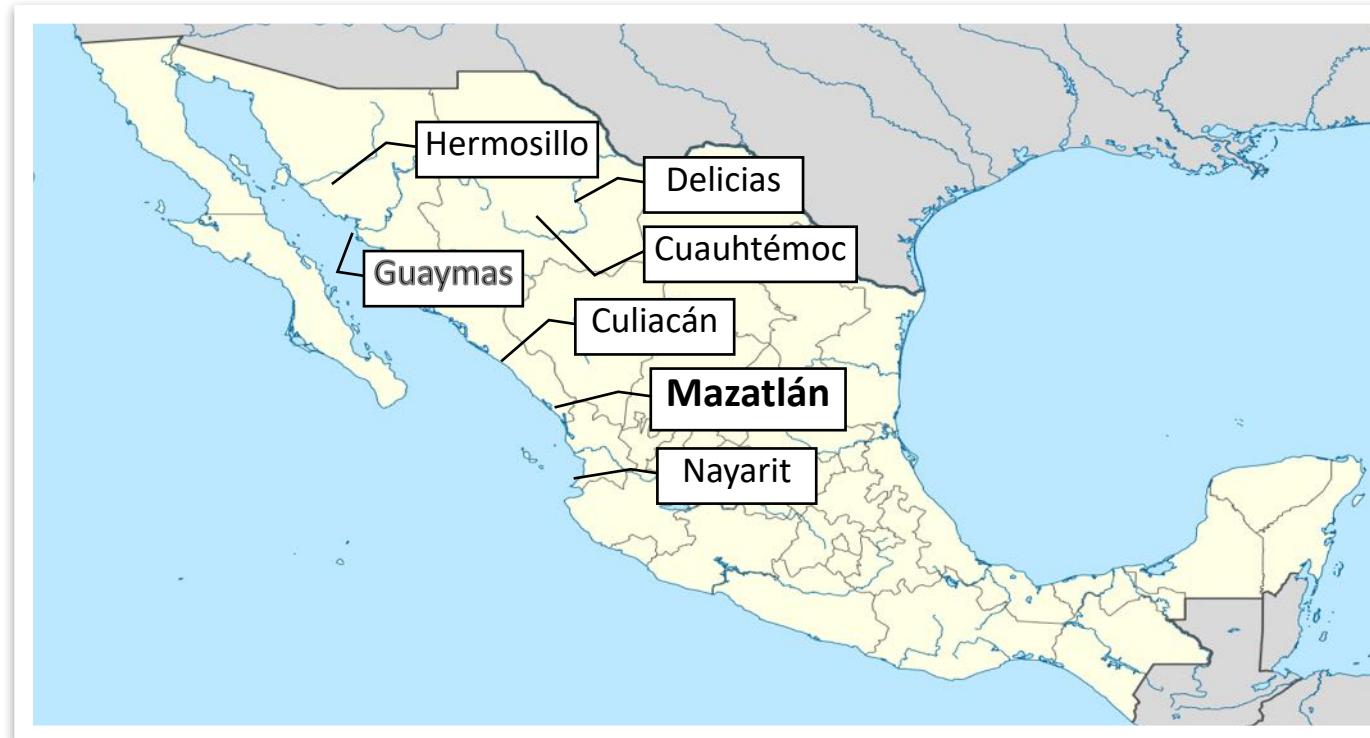
Centro de Investigación en Alimentación y Desarrollo, AC.

Centro Público de Investigación
del
Consejo Nacional de Humanidades, Ciencias y Tecnologías



Misión

Somos un centro de reflexión crítica que genera conocimiento, innova, transfiere tecnología y forma recursos humanos especializados en alimentos, nutrición, salud pública, desarrollo regional y recursos naturales, vinculado con la sociedad.





CIAD, A.C. Coordinación Regional Mazatlán

Laboratorios

- Laboratorio de Bacteriología
- Laboratorio de Genómica Microbiana
- Laboratorio de Diagnóstico Molecular
- Colección de Microorganismos de Importancia Acuática



CCCGGTAAT
LABORATORIO
ACGCDEGACC
AGENÓMICAC
MICROBIANA
GTAACTGATCC

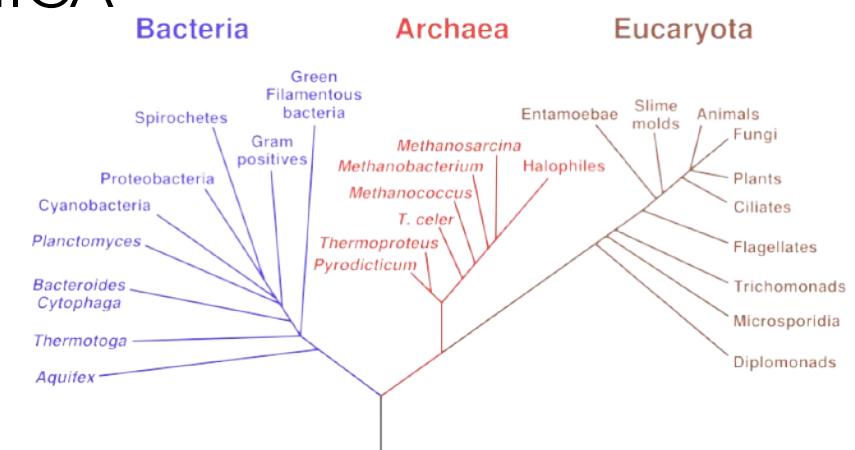
Contenido de la plática

- ¿Cómo conocer la microbiota no cultivable?
- ¿Cuántos microorganismos procariontes existen?
- ¿Cómo descubrir nuevos grupos de microorganismos?
- Ejemplos
 - Microbiota de ostiones (vibrios)
 - Diagnóstico de una infección en el líquido cefalorraquídeo

Problema con la diversidad bacteriana

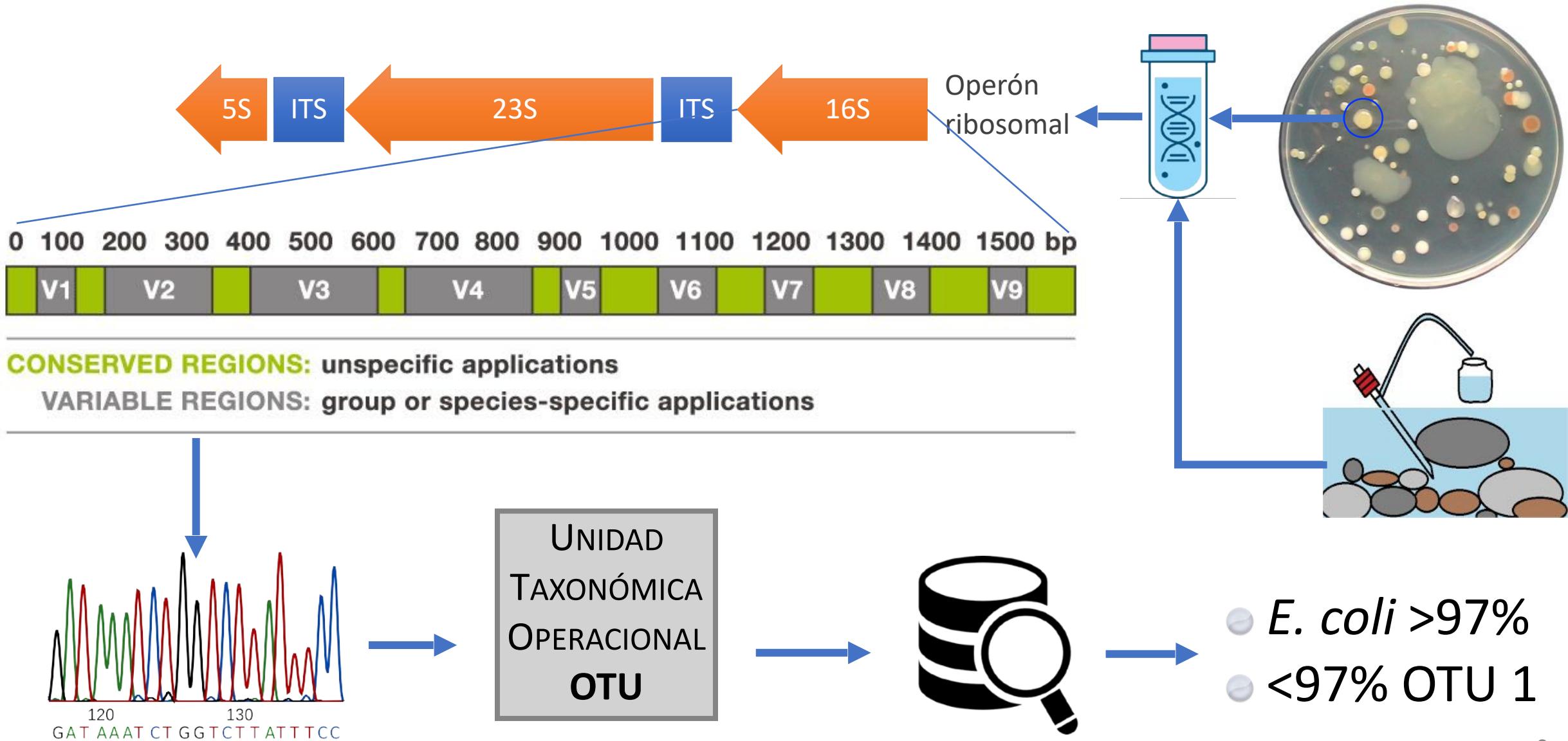


- Evaluación de la diversidad tradicionalmente mediante técnicas microbiológicas tradicionales, cultivo dependientes.
- Aproximadamente, **1-6% de las bacterias son cultivables**¹.
- No cultivables?
 - Análisis de ADN en la muestra -> METAGENÓMICA



¹Amann et al. 1990. AEM 56:1919-25. Wang et al. PeerJ. 2020; 8: e10060

Clasificación taxonómica por secuenciación del 16S

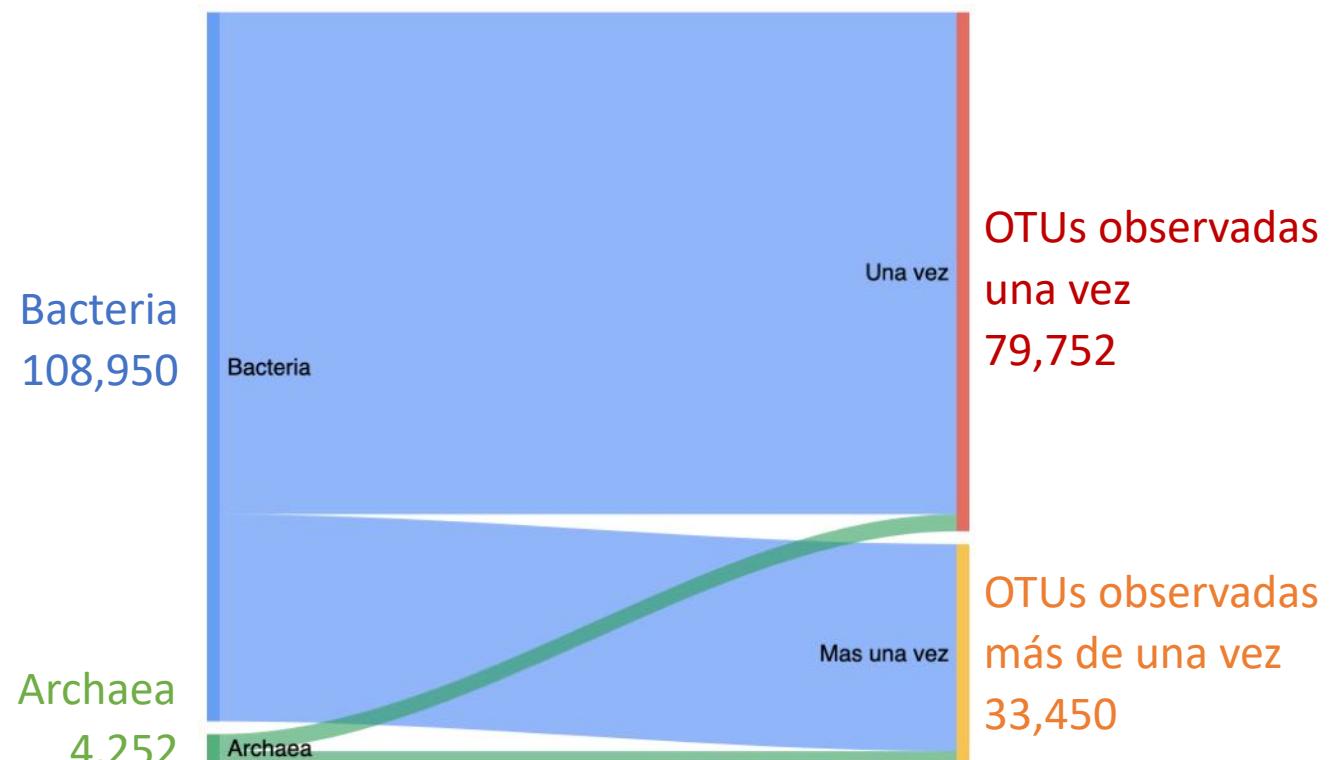


¿Cuántos microorganismos procariontes existen?

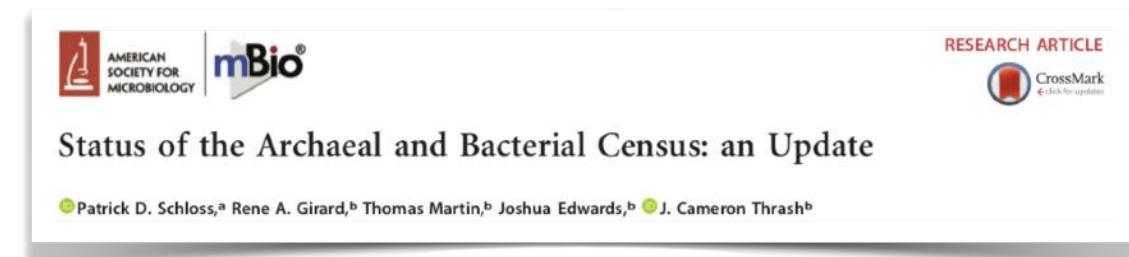
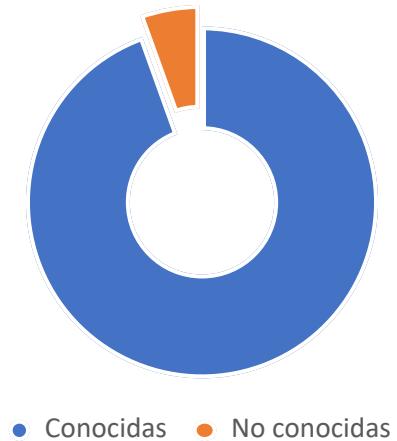
Bacterias y arqueas

Diversidad con base en el 16S

	Secuencias 16S conocidas	OTUs observadas más de una vez	Aislado cultivados
Bacteria	94.5 %	29.2 %	16.9 %
Archaea	95.1 %	38.5 %	13.1 %

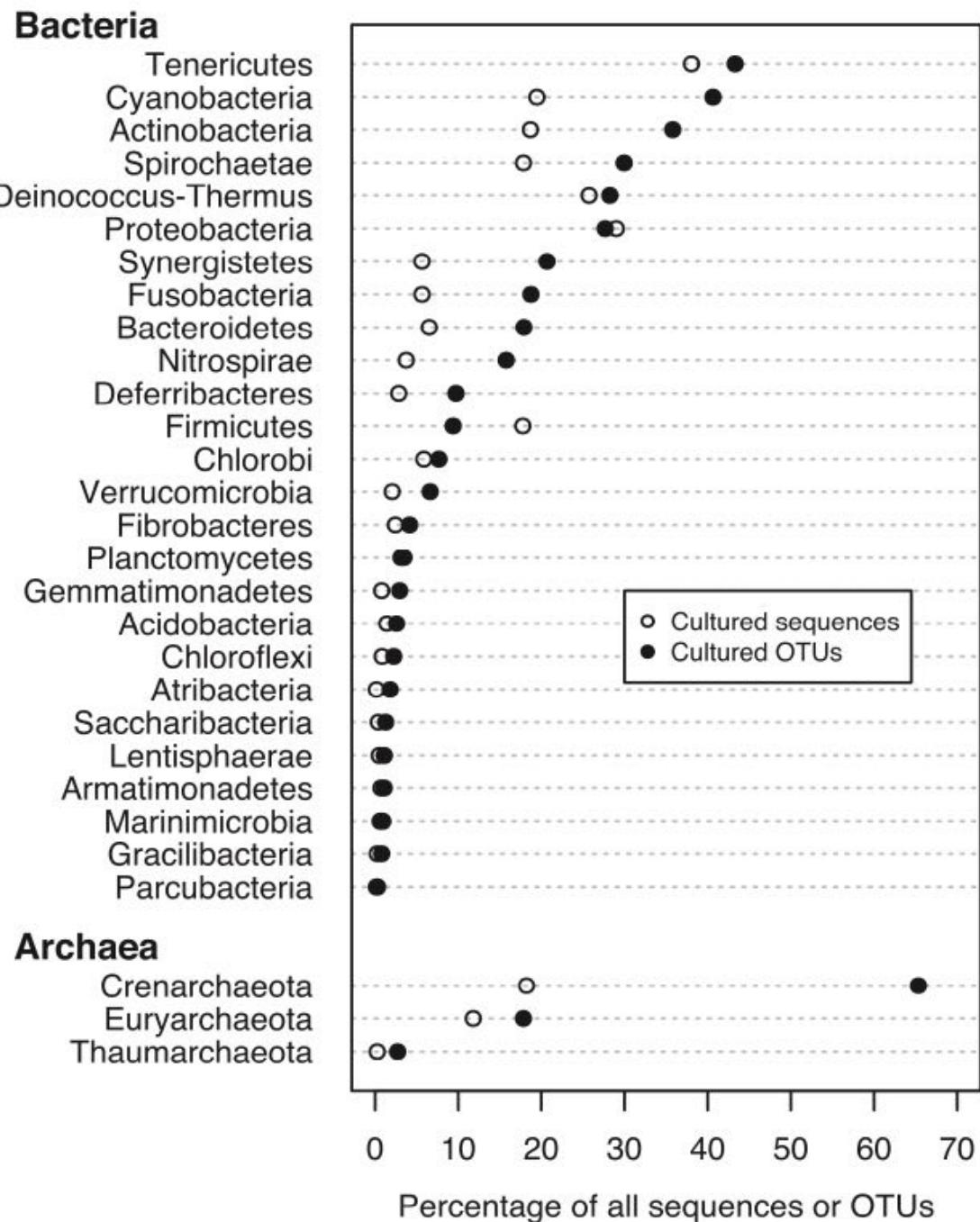
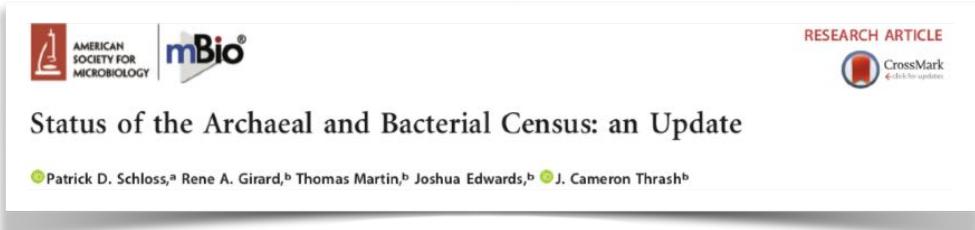


Nuevas secuencias 16S

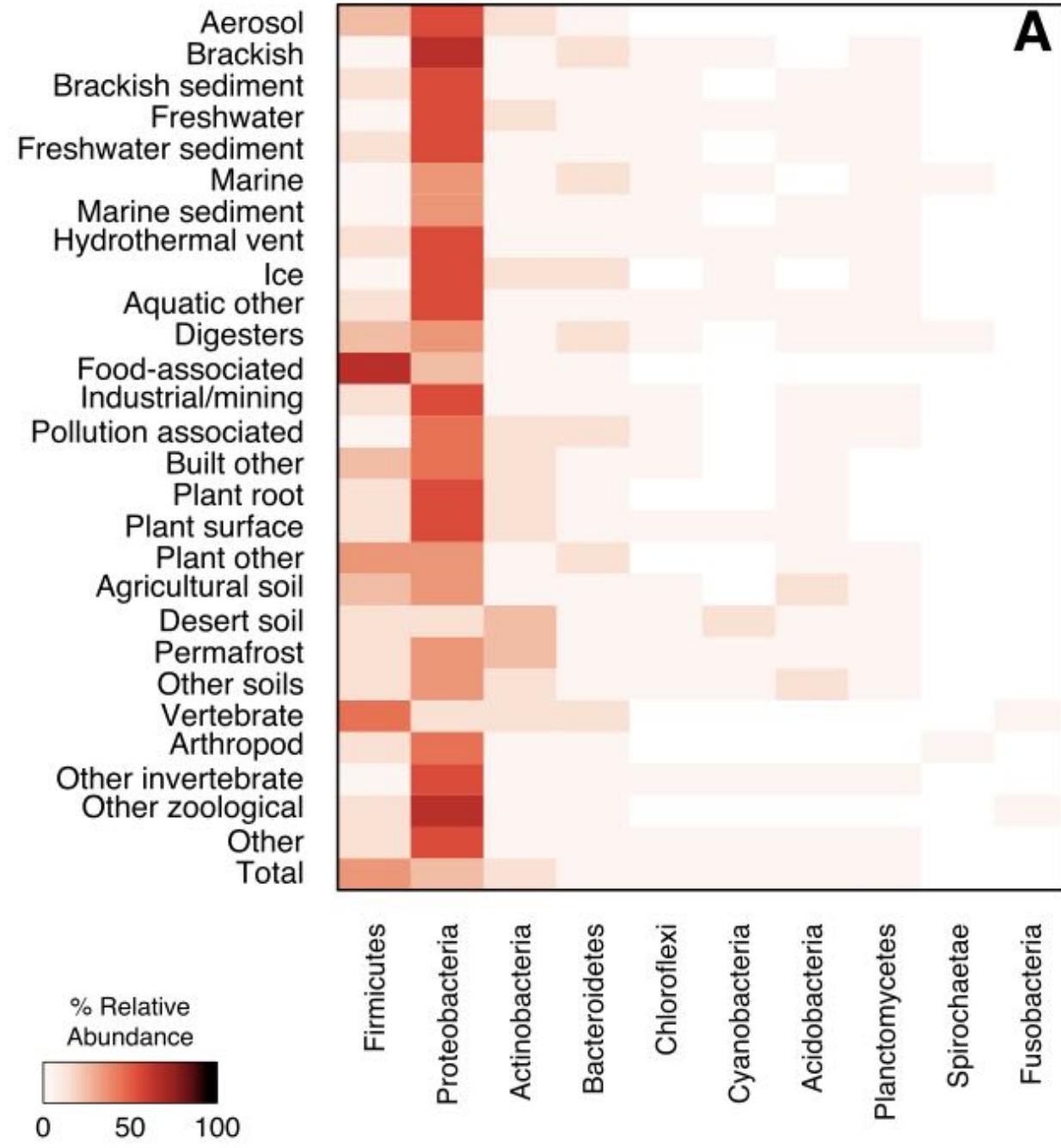


¿Qué porcentaje de OTUs tienen al menos un organismo cultivable?

- % secuencias que tienen organismo cultivado
- % OTUs con secuencias de organismos cultivados



¿Quiénes están en los diferentes ambientes?



Status of the Archaeal and Bacterial Census: an Update

Patrick D. Schloss,^a Rene A. Girard,^b Thomas Martin,^b Joshua Edwards,^b J. Cameron Thrash^b

¿Cuántos microorganismos procariontes existen?

- 16S ribosomal RNA amplicon sequences in the V4 hypervariable region obtained from
- 2.2–4.3 million full-length OTUs worldwide 492 studies worldwide
- **0.8 – 1.6 M (10^6) taxones microbianos.**

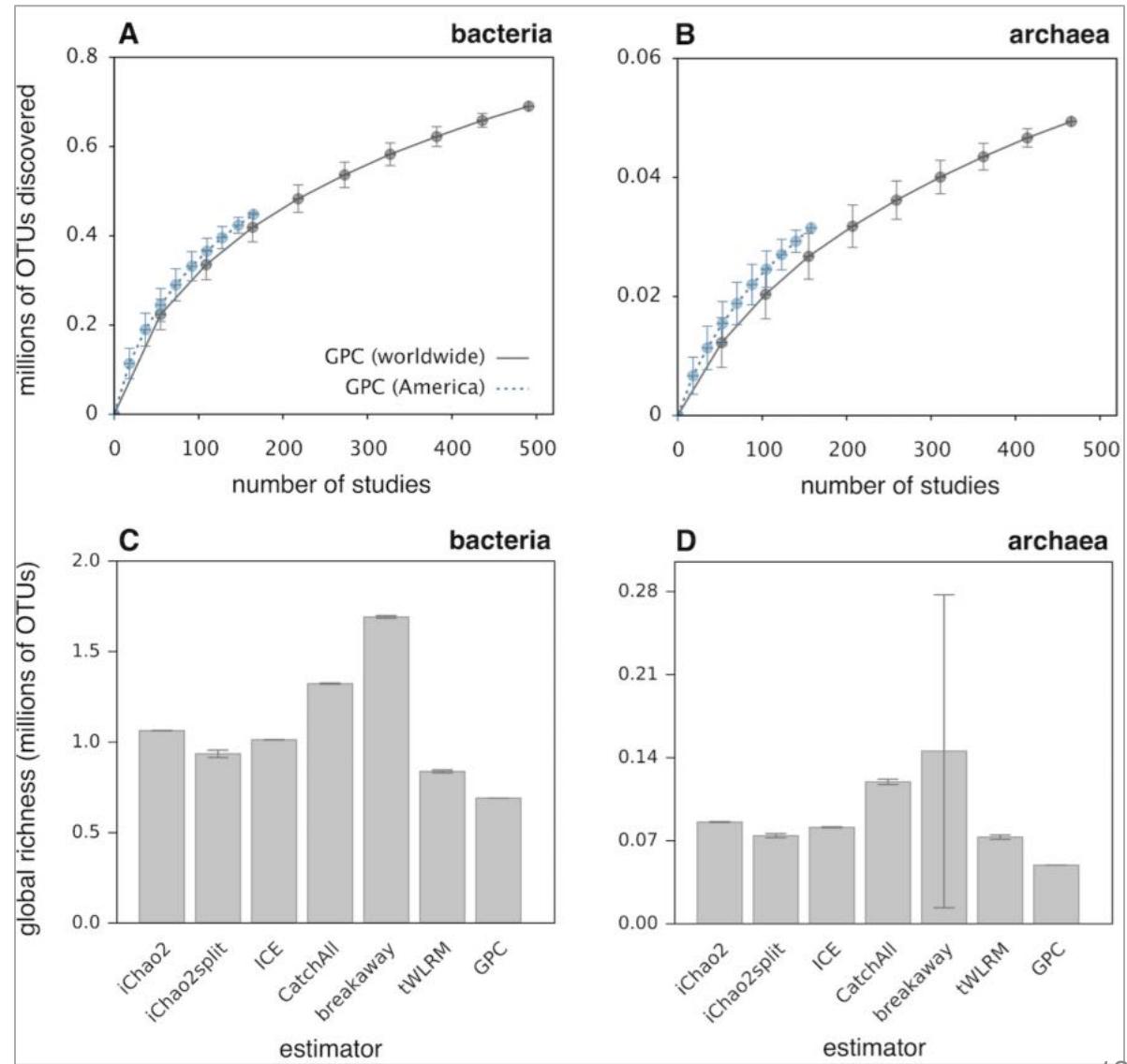
PLOS | **BIOLOGY**

RESEARCH ARTICLE

A census-based estimate of Earth's bacterial and archaeal diversity

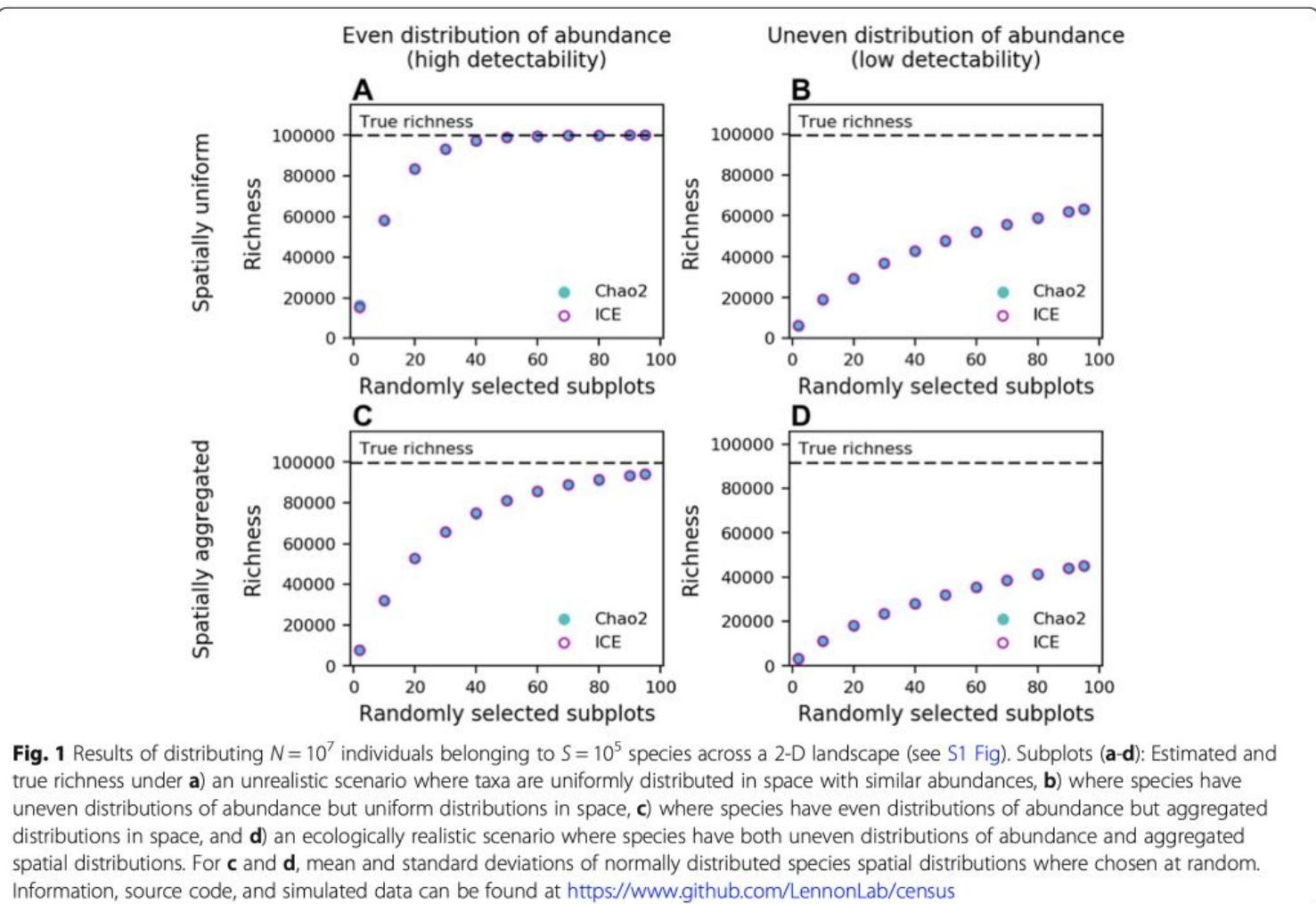
Stilianos Louca^{1,2,3,4*}, Florent Mazel^{3,5}, Michael Doebeli^{3,4,6}, Laura Wegener Parfrey^{3,4,5}

1 Department of Biology, University of Oregon, Eugene, Oregon, United States of America, 2 Institute of Ecology and Evolution, University of Oregon, Eugene, Oregon, United States of America, 3 Biodiversity Research Centre, University of British Columbia, Vancouver, Canada, 4 Department of Zoology, University of British Columbia, Vancouver, Canada, 5 Department of Botany, University of British Columbia, Vancouver, Canada, 6 Department of Mathematics, University of British Columbia, Vancouver, Canada



¿Cuántos microorganismos procariontes existen?

- 10^{12} taxones de microorganismos



Lennon and Locey *Biology Direct* (2020) 15:5
<https://doi.org/10.1186/s13062-020-00261-8>

Biology Direct

COMMENT

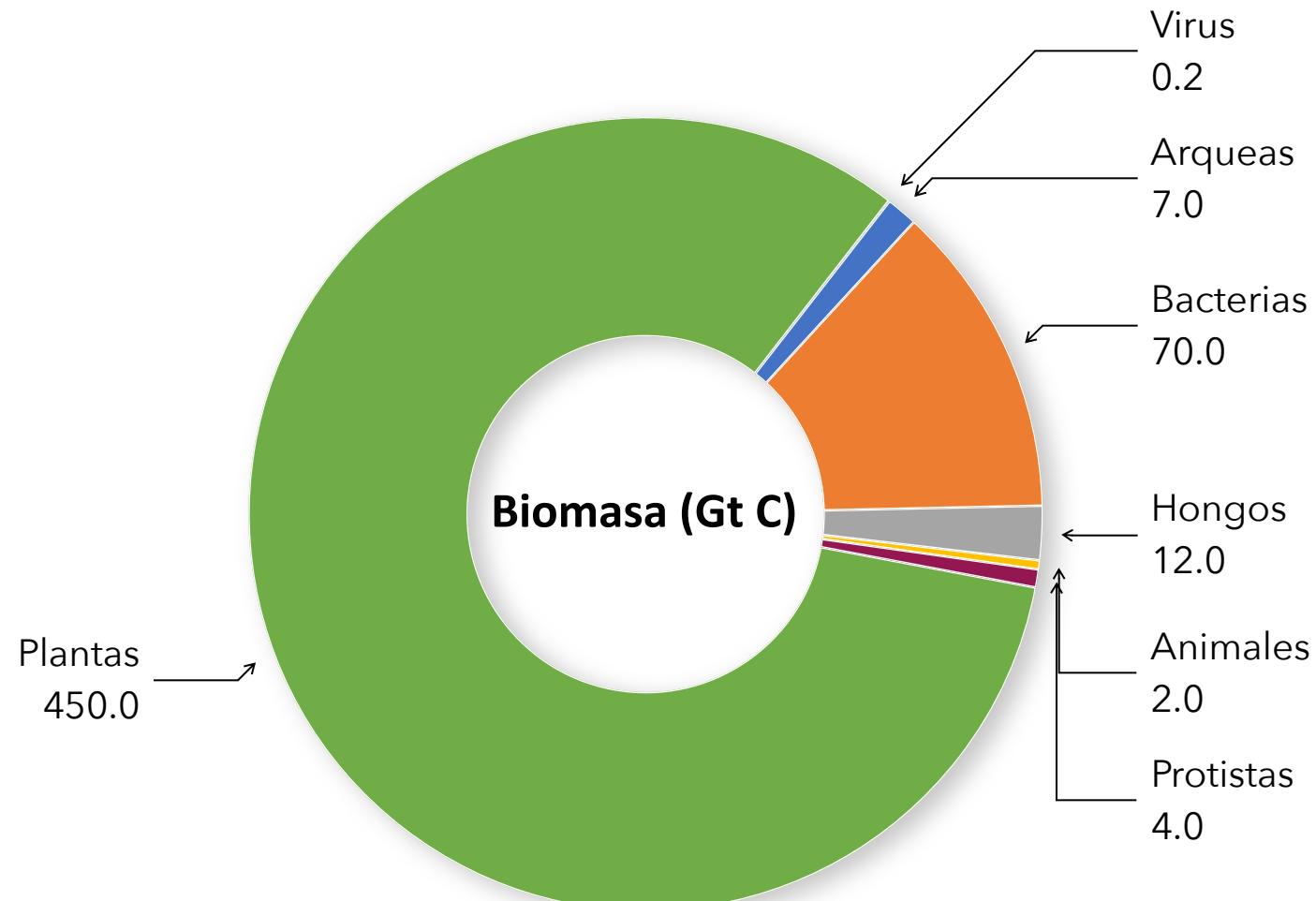
Open Access

More support for Earth's massive microbiome



Jay T. Lennon^{1*} and Kenneth J. Locey^{2*}

¿Cuál es la biomasa de los microorganismos?



PNAS

RESEARCH ARTICLE | BIOLOGICAL SCIENCES | 8

f t in e

The biomass distribution on Earth

Yinon M. Bar-On, Rob Phillips, and Ron Milo
Edited by Paul G. Falkowski, Rutgers, The State University of New Jersey, New Brunswick, NJ, and approved April 13, 2018 (received for review July 3, 2017)

May 21, 2018 | 115 (25) 6506-6511 | <https://doi.org/10.1073/pnas.1711842115>

¿Cómo descubrir nuevos grupos?

Metagenómica shotgun

Metagenomics

"the application of modern genomics techniques to the study of communities of microbial organisms directly in their natural environments, bypassing the need for isolation and lab cultivation of individual species"

Chen, K.; Pachter, L. (2005). PLoS Computational Biology 1 (2): e24.

Metagenomics is the study of metagenomes, genetic material recovered directly from environmental samples.

Wikipedia

Aproximaciones Metagenómicas

- Caracterización por medio de un gen (*Taxonomic fingerprinting*)
 - 16S rRNA
- Secuenciación de todo el ADN
 - ADN metagenómica. ¿Quiénes son y qué son capaces de hacer?
 - RNA metatranscriptómica. ¿Que están haciendo?

contig assembly

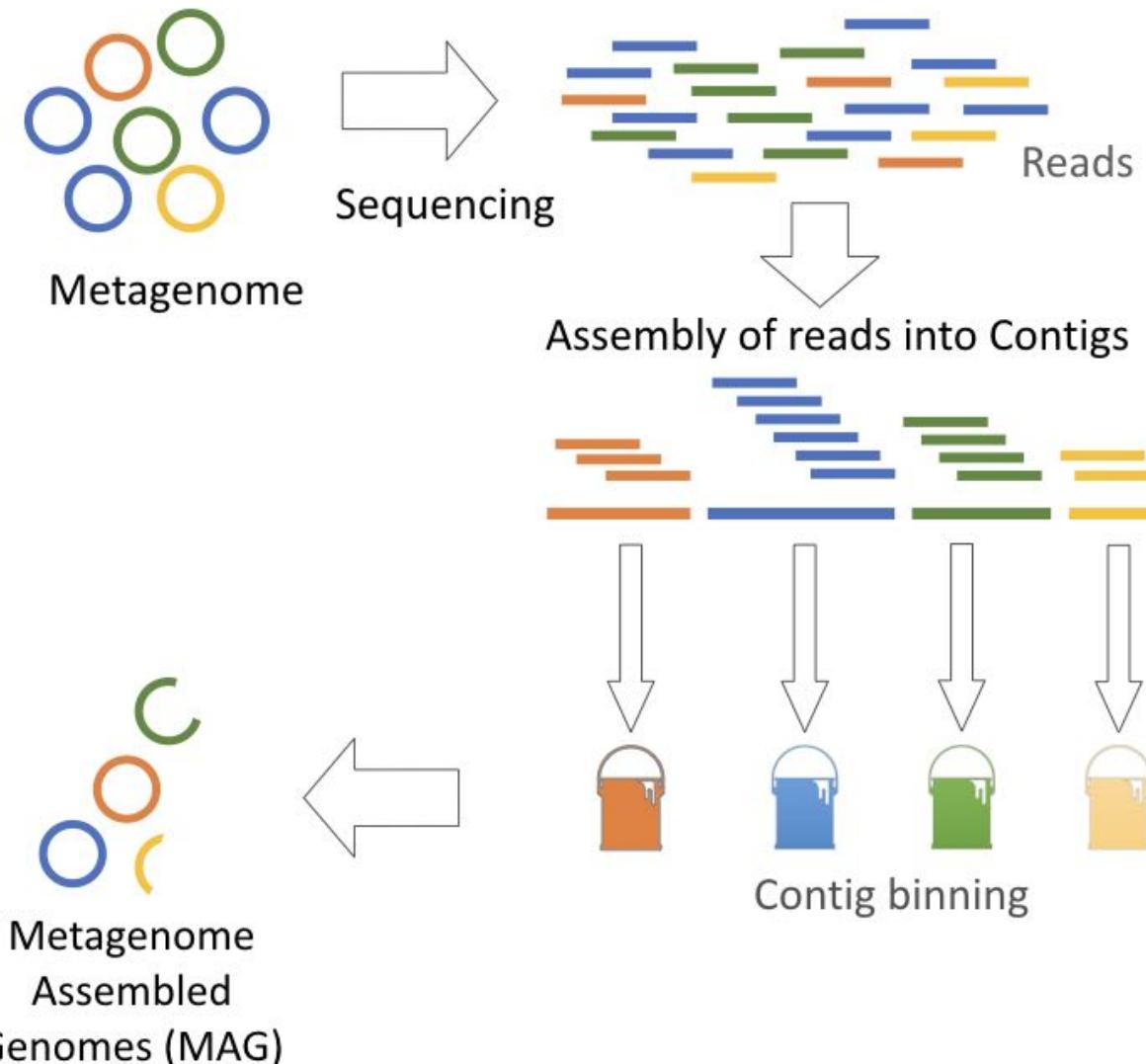
Reads

AAGCCTGCGGTTA	CAAAT
AGCCTGCGGTTACTG	AAAT
TGC GGTC ACTGC	AAT
CGGTTACTGGCC	
GGTTTCTGGCCAA	
GTTACTGGCCAAAT	= 9 reads

contig coverage

AAGCCTGCGGTTACTGGCCAAAT	= 23bp
1222233456665553344544	= 4.04X

Metagenome Assembled Genome (MAG) Binning

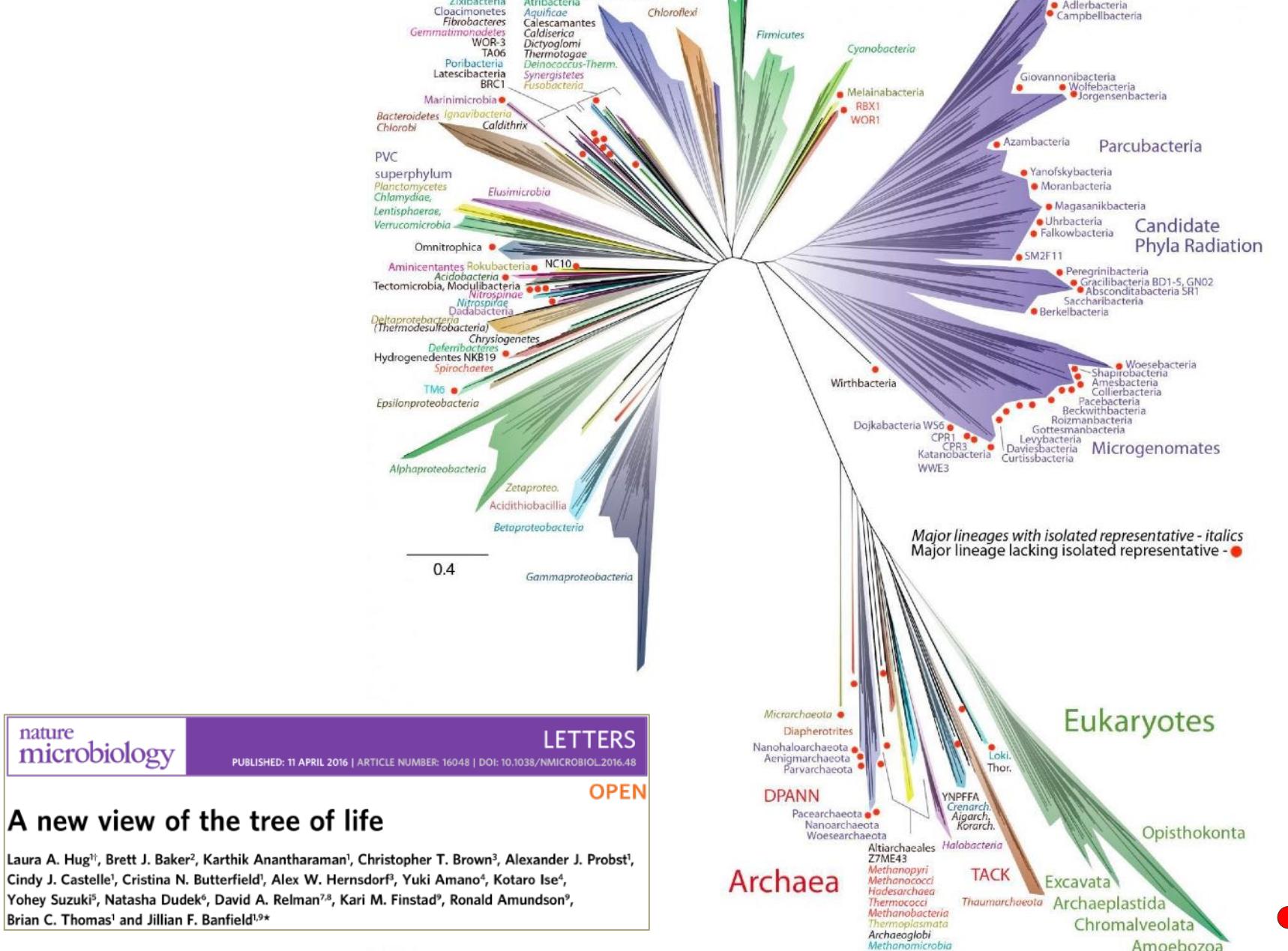


MAG Validation

Bacterial single-copy core gene (BSCG) collections*

- Completeness: > 90%
- Redundancy: < 10%

* Campbell et al. 2013. PNAS 110:5540-45



Candidate Phyla Radiation (CPR)

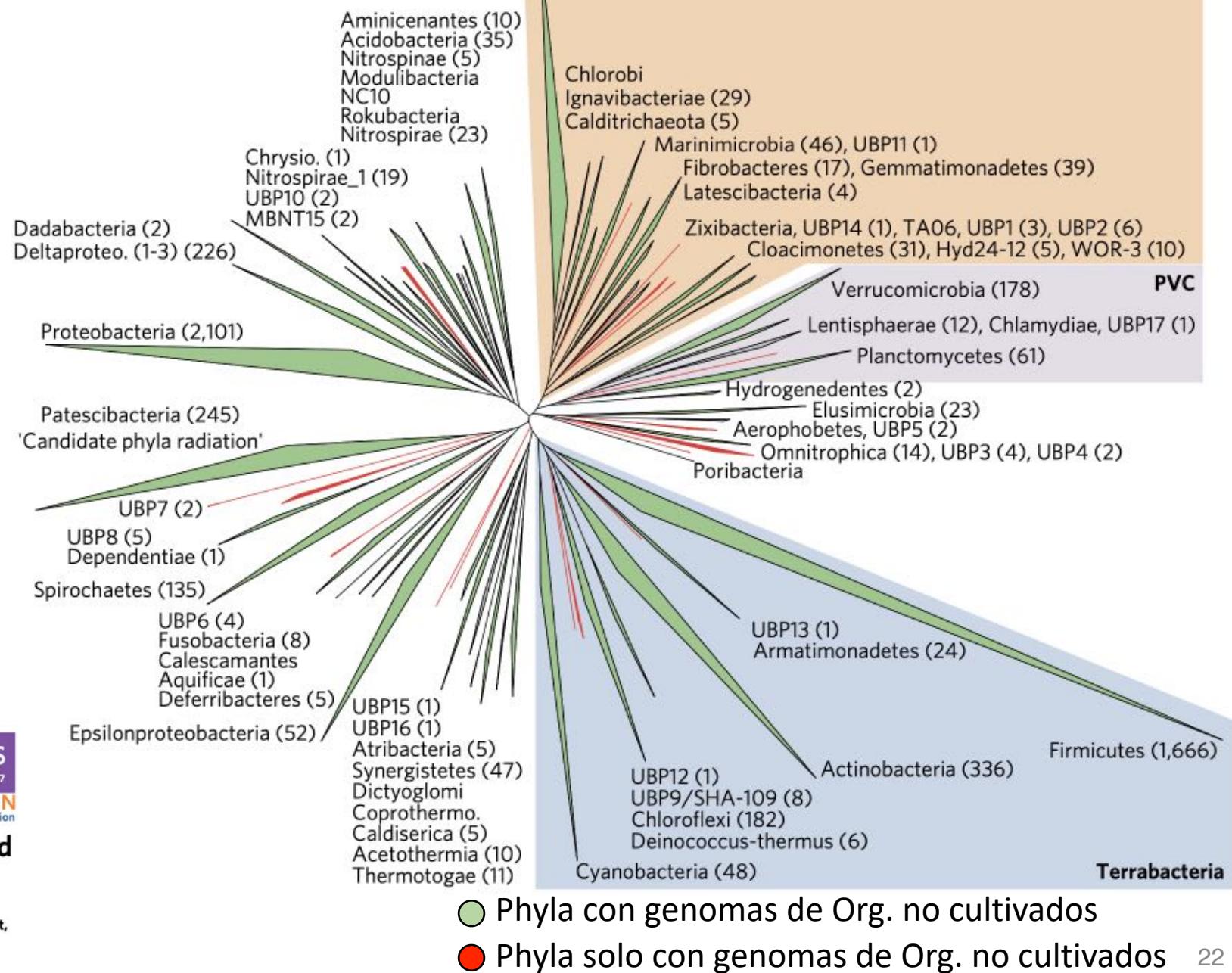
- Pequeños genomas
- Limitaciones metabólicas
- Obligados fermentadores

nature > letters > article
Published: 15 June 2015
Unusual biology across a group comprising more than 15% of domain Bacteria
Christopher T. Brown, Laura A. Hug, Brian C. Thomas, Itai Sharon, Cindy J. Castelle, Andrea Singh, Michael J. Wilkins, Kelly C. Wrighton, Kenneth H. Williams & Jillian F. Banfield
Nature 523, 208–211 (2015) | Cite this article

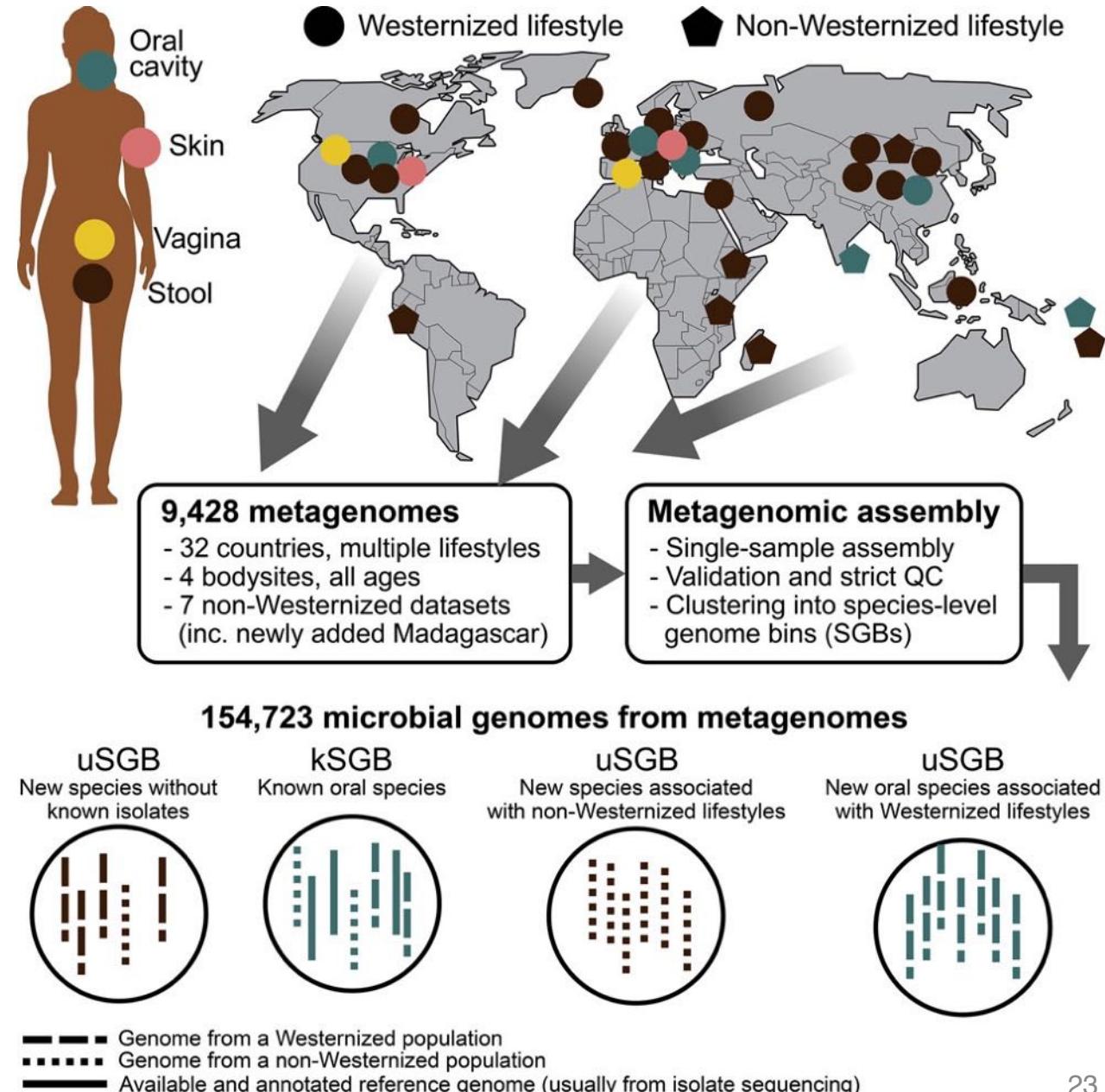
● Filos sin aislados cultivables

0.1

- 7,903 MAGs a partir de
- 1,500 metagenomas
- > 50% completos
- Phyla candidatos:
 - 17 Bacteria
 - 3 Archaea



150,000 MAGs



Edoardo Pasolli,¹ Francesco Asnicar,^{1,8} Serena Manara,^{1,8} Moreno Zolfo,^{1,8} Nicolai Karcher,¹ Federica Armanini,¹ Francesco Beghini,¹ Paolo Manghi,¹ Adrian Tett,¹ Paolo Ghensi,¹ Maria Carmen Collado,² Benjamin L. Rice,³ Casey DuLong,⁴ Xochitl C. Morgan,⁵ Christopher D. Golden,⁴ Christopher Quince,⁶ Curtis Huttenhower,^{4,7} and Nicola Serafini^{1,9,*}

Ensenada, B.C. September 9-13, 2019

Microbiota de ostiones

Uso de NGS para detectar vibrios de importancia médica

Bacteria in oysters

- Strong emphasis in Vibrios
 - *V. cholerae*
 - *V. parahaemolyticus*
 - *V. vulnificus*
- Culture-dependent methods identified:
 - *Aeromonas, Alteromononas, Pseudomonas, Micrococcus, Vibrio, etc.*
- Little known about the whole microbiota present

Material availability

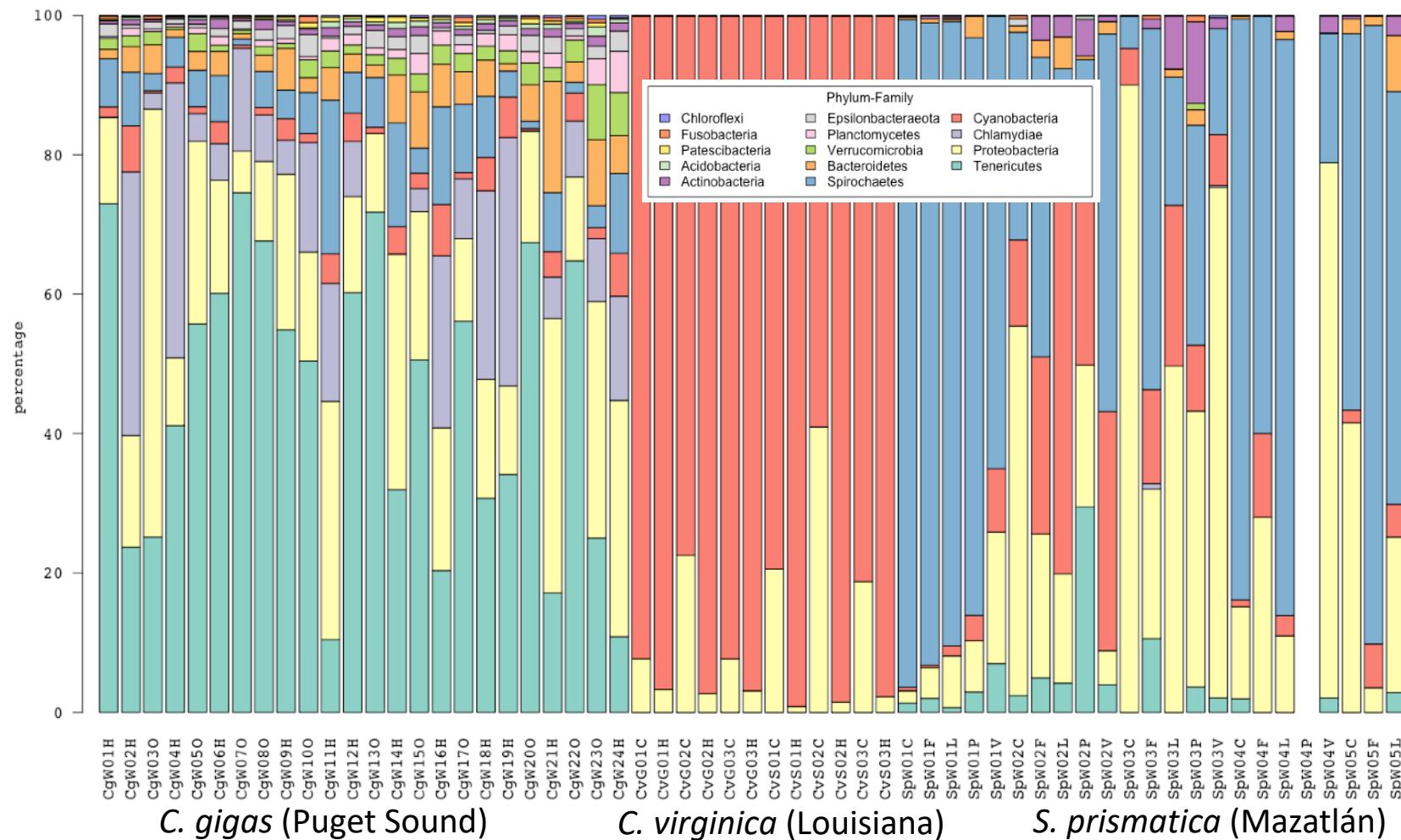
- Zhen, L & Wang L. 2017
 - *Crassostreae gigas* (whole) 2014
 - Puget Sound, USA
 - MG-RAST, Illumina
- King, G et al. 2012 (PLOS ONE)
 - *Crassostreae virginica* (gut, stomach) 2010
 - Mississippi delta, Louisiana, USA
 - MG-RAST, 454
- Luis, I. et al.
 - *Striostrea prismatica* (whole), 2015-16
 - Mazatlán, Sinaloa, México
 - Ion Torrent
- Texas A&M
 - *Crassostreae* sp. (gut), 2016
 - Texas, USA
 - EMBL, Illumina HiSeq



16S amplicon fingerprinting

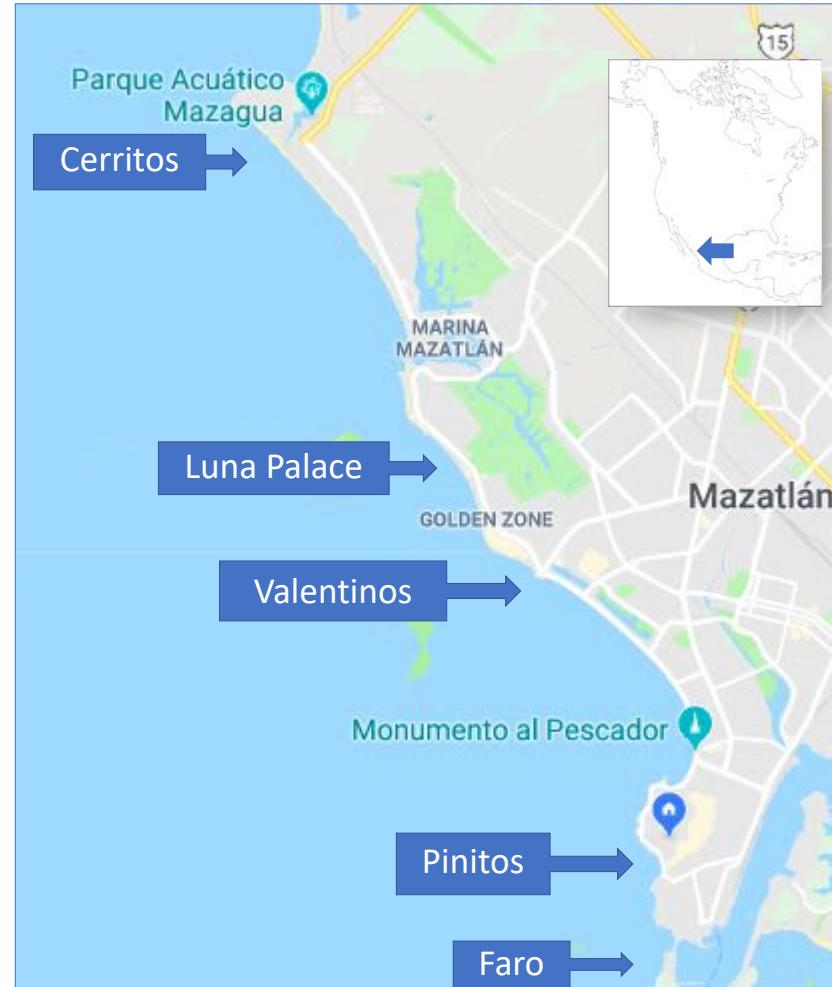
C. gigas (Puget Sound), *C. virginica* (Louisiana),
and *S. prismatica* (Mazatlán)

Principal phyla found (> 95 %)

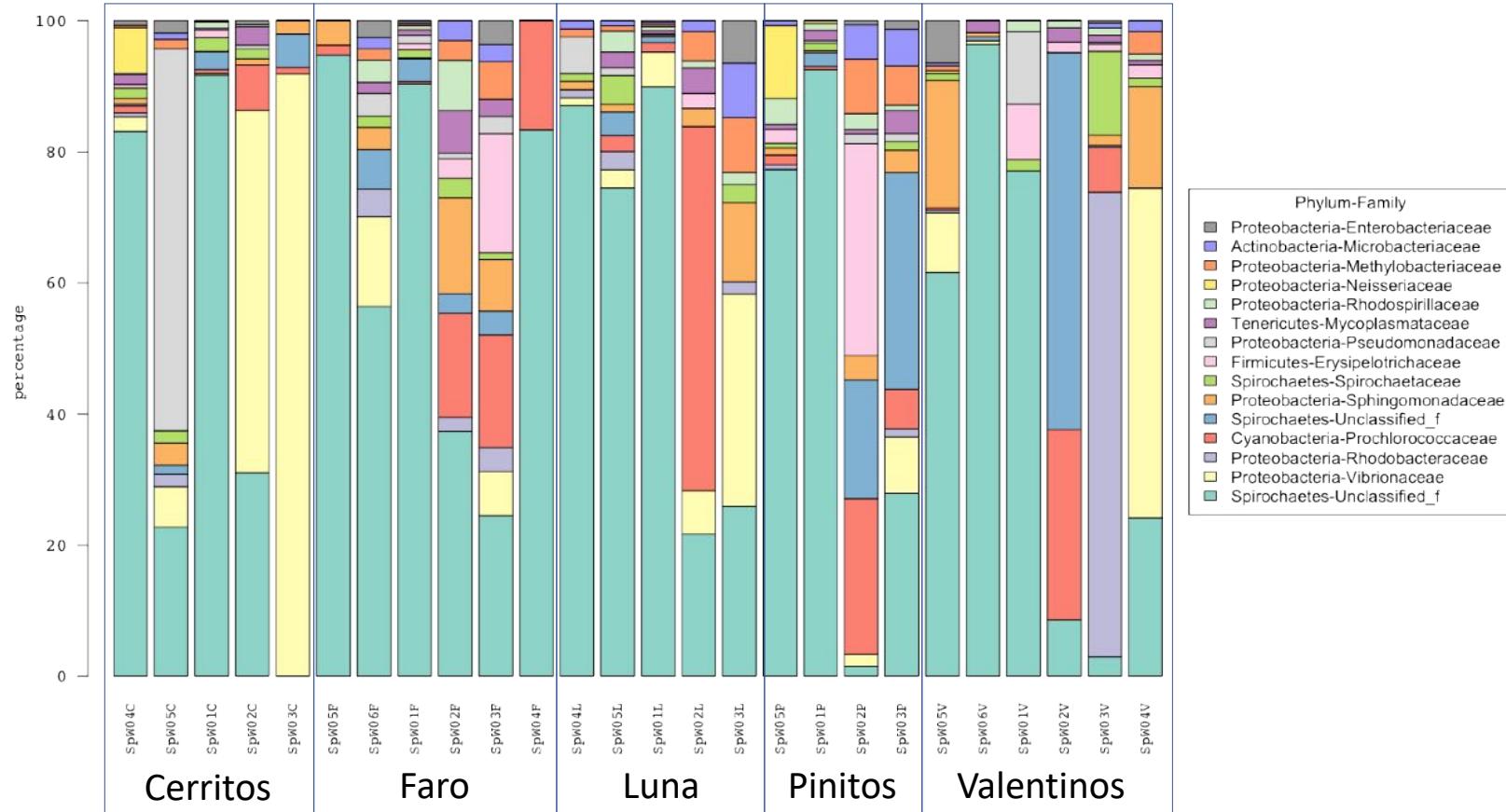


Mazatlán

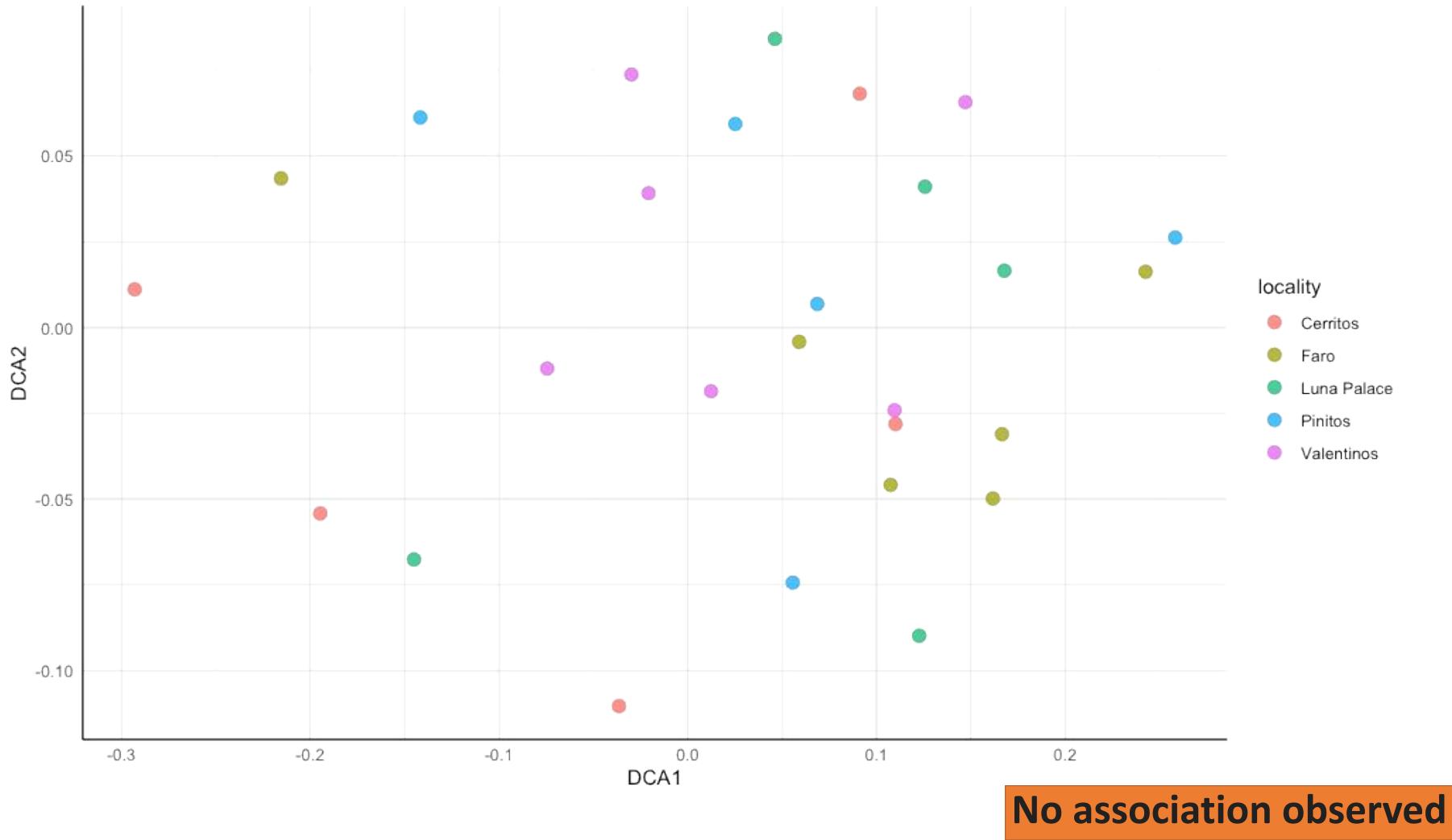
- *S. prismatica*
- Whole oyster
- 16S V3, Ion Torrent
- Jan, Feb, May, Sep, Dec
- 2016



Differences between Mazatlan localities (families)

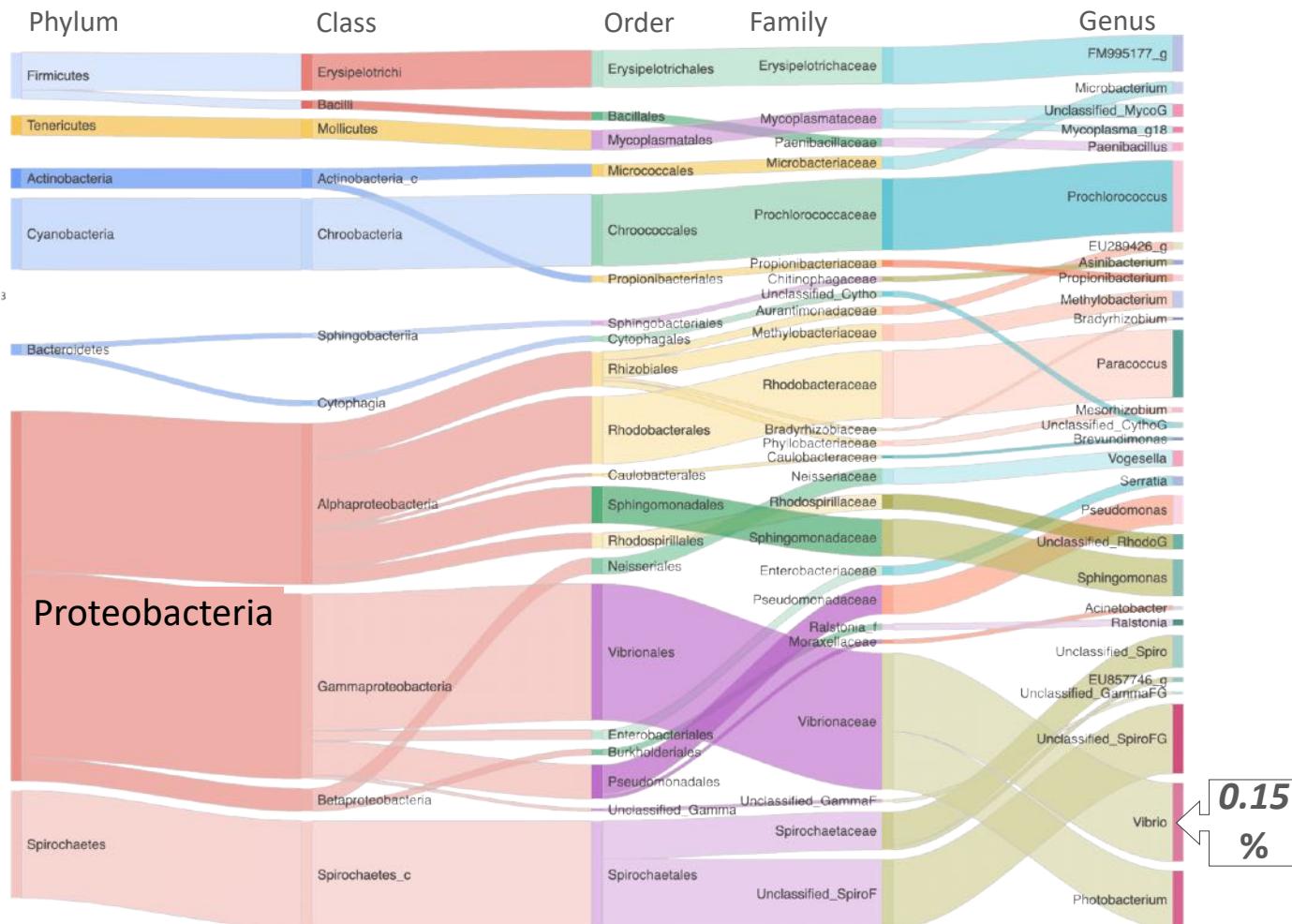
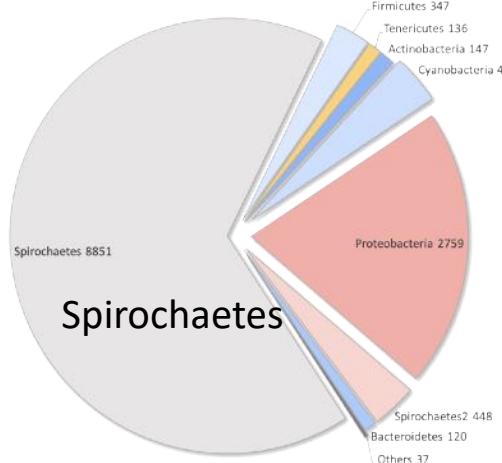


Detrended correspondence analysis



Mazatlán

All samples



Puget Sound, WA



AMERICAN SOCIETY FOR MICROBIOLOGY

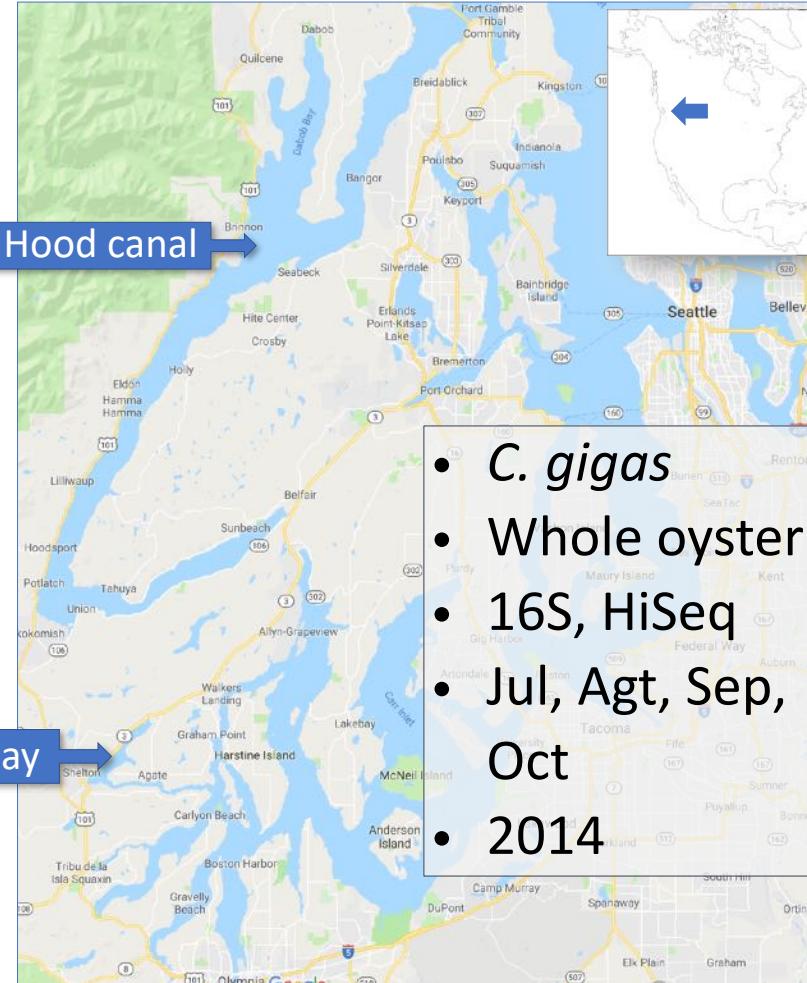


Metagenomic 16S rRNA Sequencing Analysis of Pacific Oyster (*Crassostrea gigas*) Microbiota from the Puget Sound Region in the United States

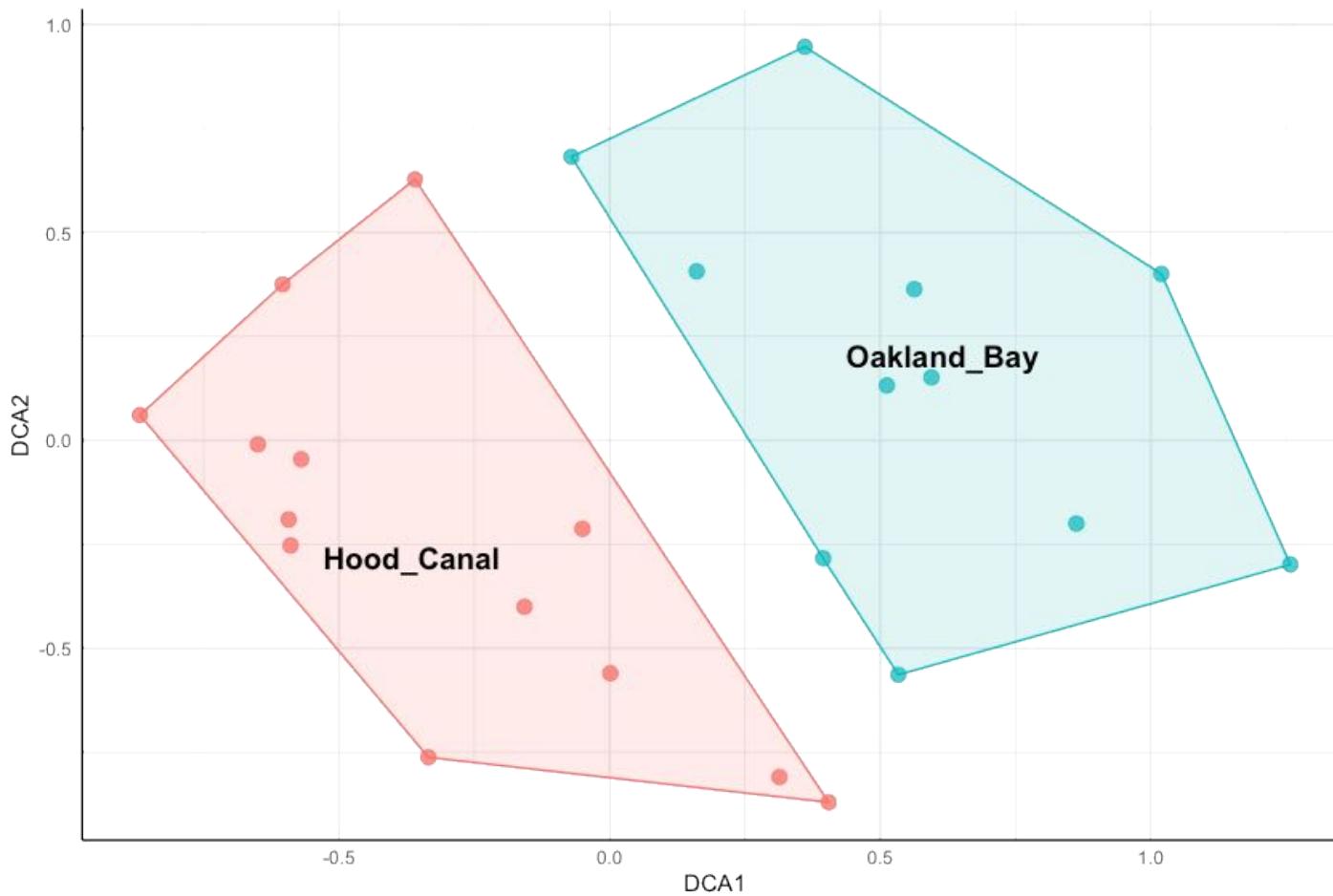
Zhen Li,^a Leyi Wang^b

Washington State Department of Health, Public Health Laboratories, Shoreline, Washington, USA^a; Ohio Department of Agriculture, Animal Disease Diagnostic Laboratory, Reynoldsburg, Ohio, USA^b

ABSTRACT This is the first study analyzing Pacific oyster microbiota in the Puget Sound estuarine system using a next-generation sequencing method. Taxonomic analysis indicated that *Tenericutes*, *Chlamydiae*, *Proteobacteria*, and *Firmicutes* were the most abundant phyla. Small numbers of operational taxonomic units (OTUs) belonging to the *Vibrio* genus were detected in all the oyster microbiome samples.



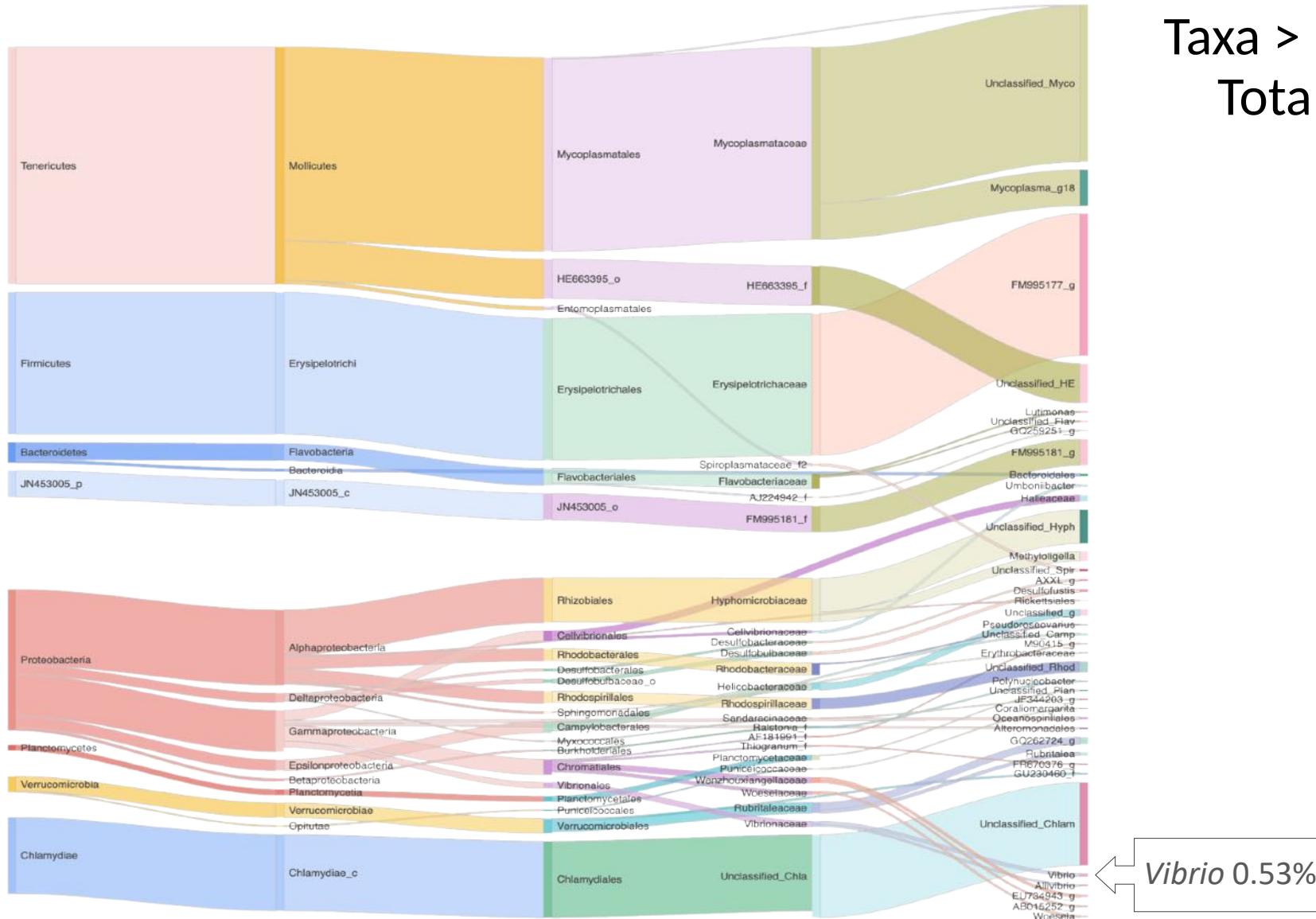
Detrended correspondence analysis



Oakland Bay

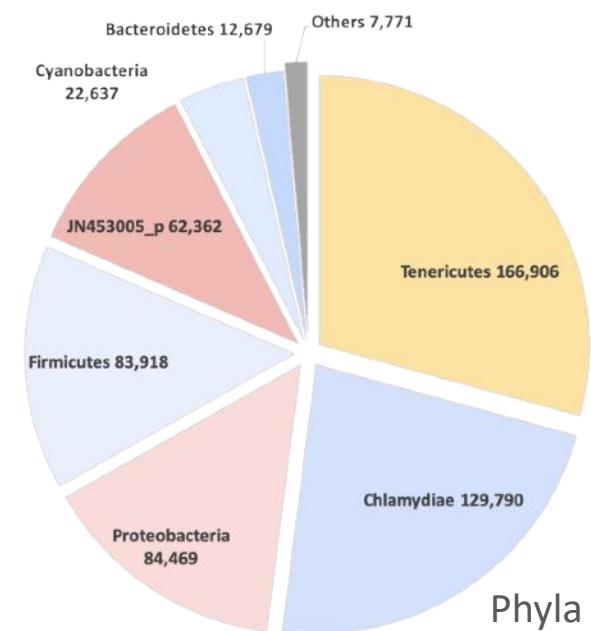
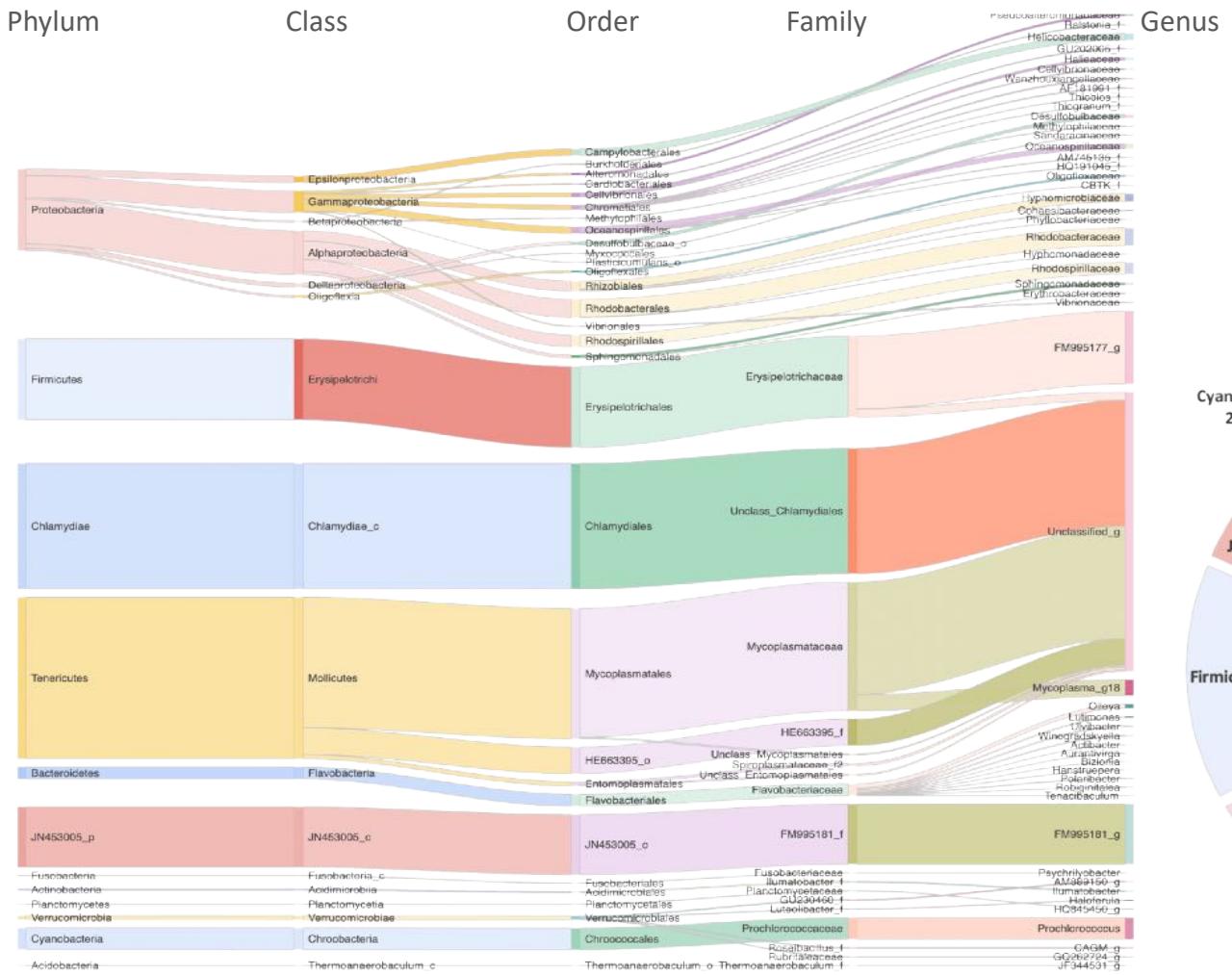
Taxa > 100 hits

Total 52,268



Hood canal

Taxa > 400 hits
Total 727,076



Phylum

Class

Order

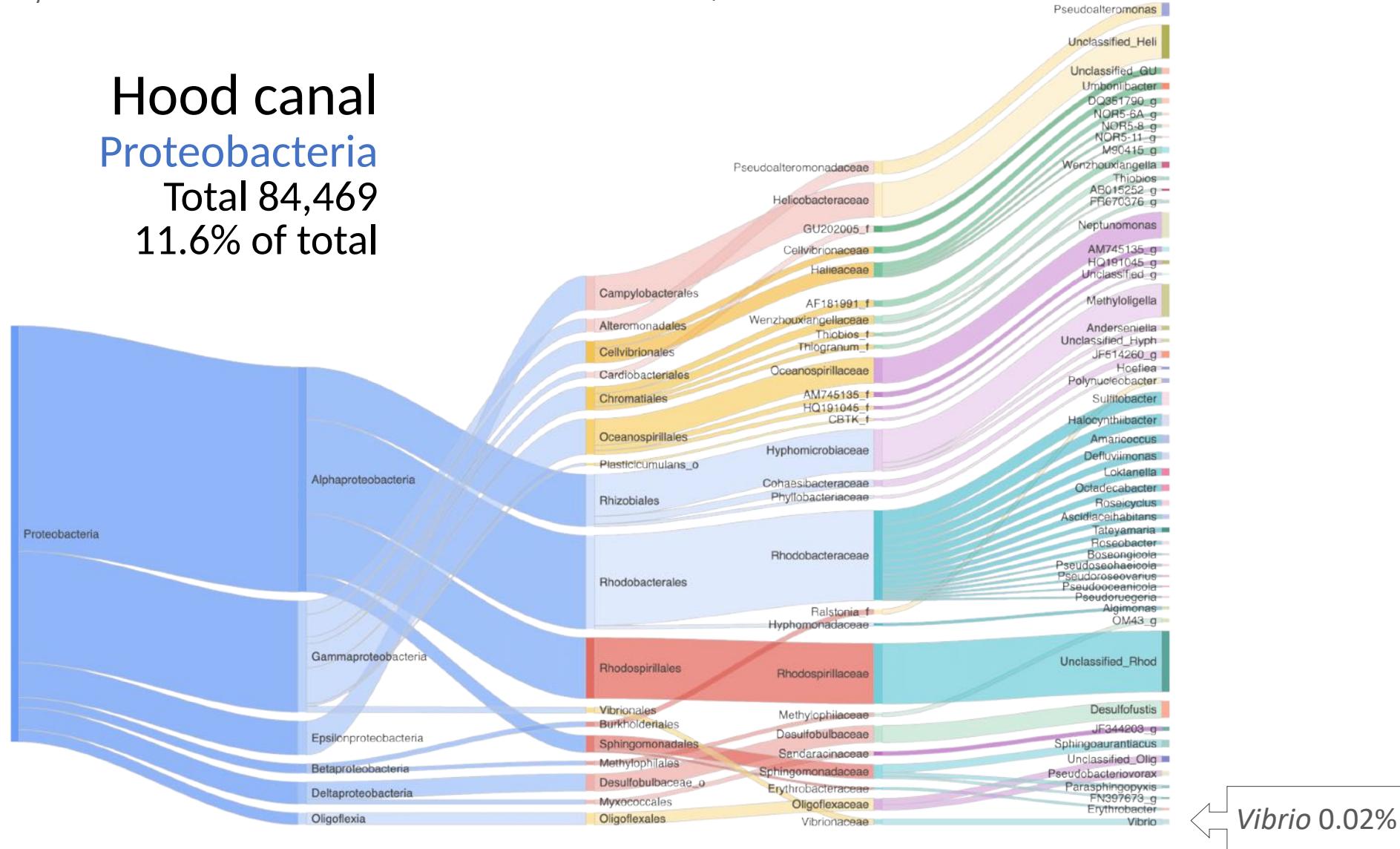
Family

Genus

Hood canal

Proteobacteria

Total 84,469
11.6% of total



Shotgun metagenomics

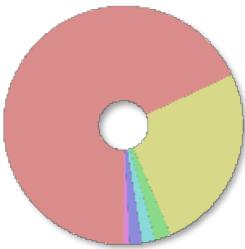
Texas oysters and surrounding water

Texas

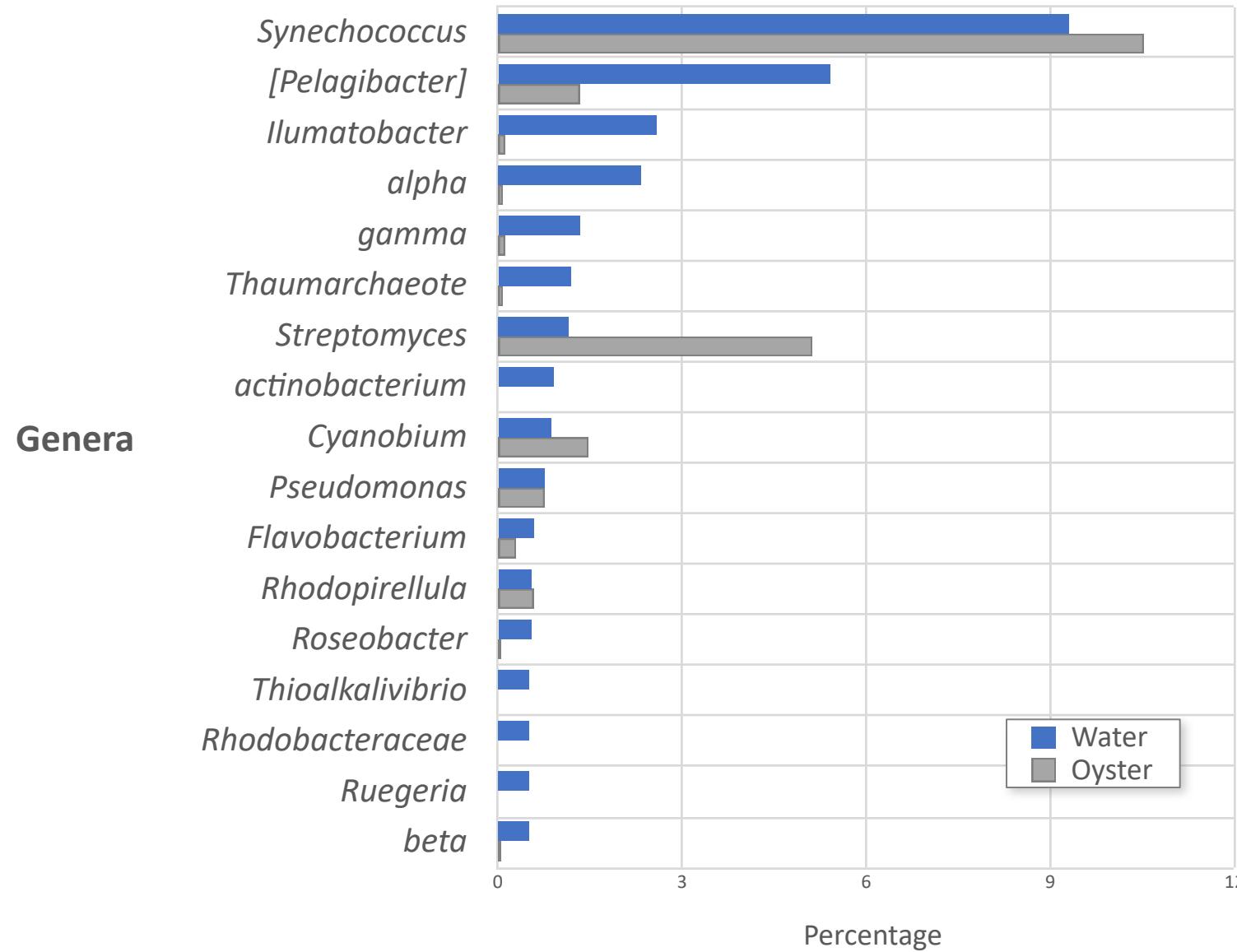
- *Crassostrea* sp.
 - Gut content
 - Metagenome
 - HiSeq (48 M Seqs/
sample)
 - 2016



Krona charts

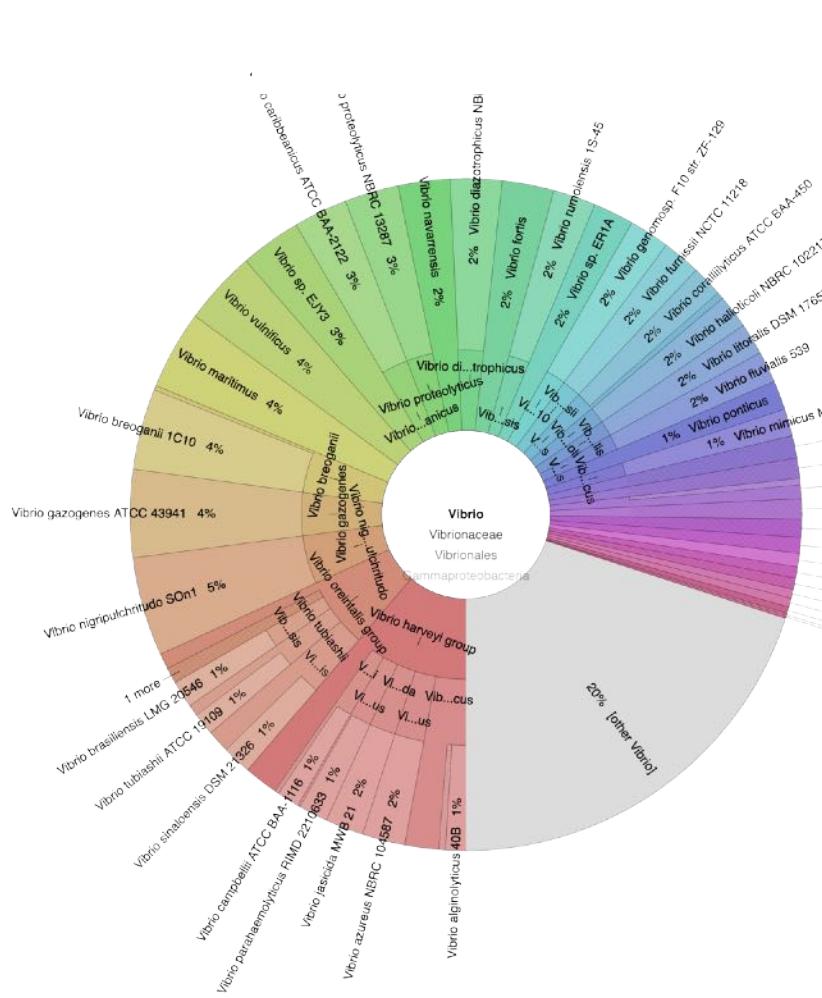


Galveston Water / Oysters

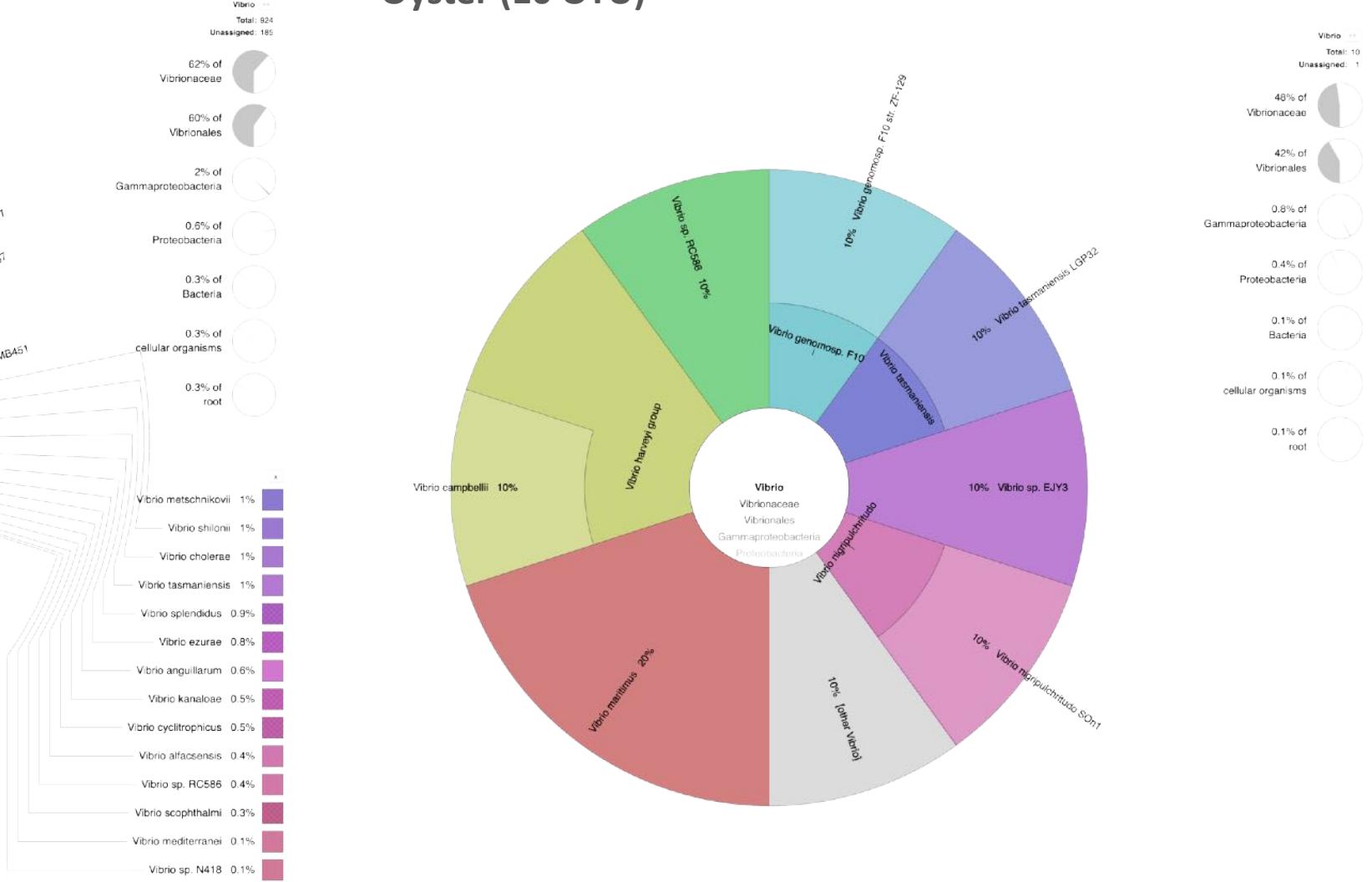


Galveston Vibrio diversity

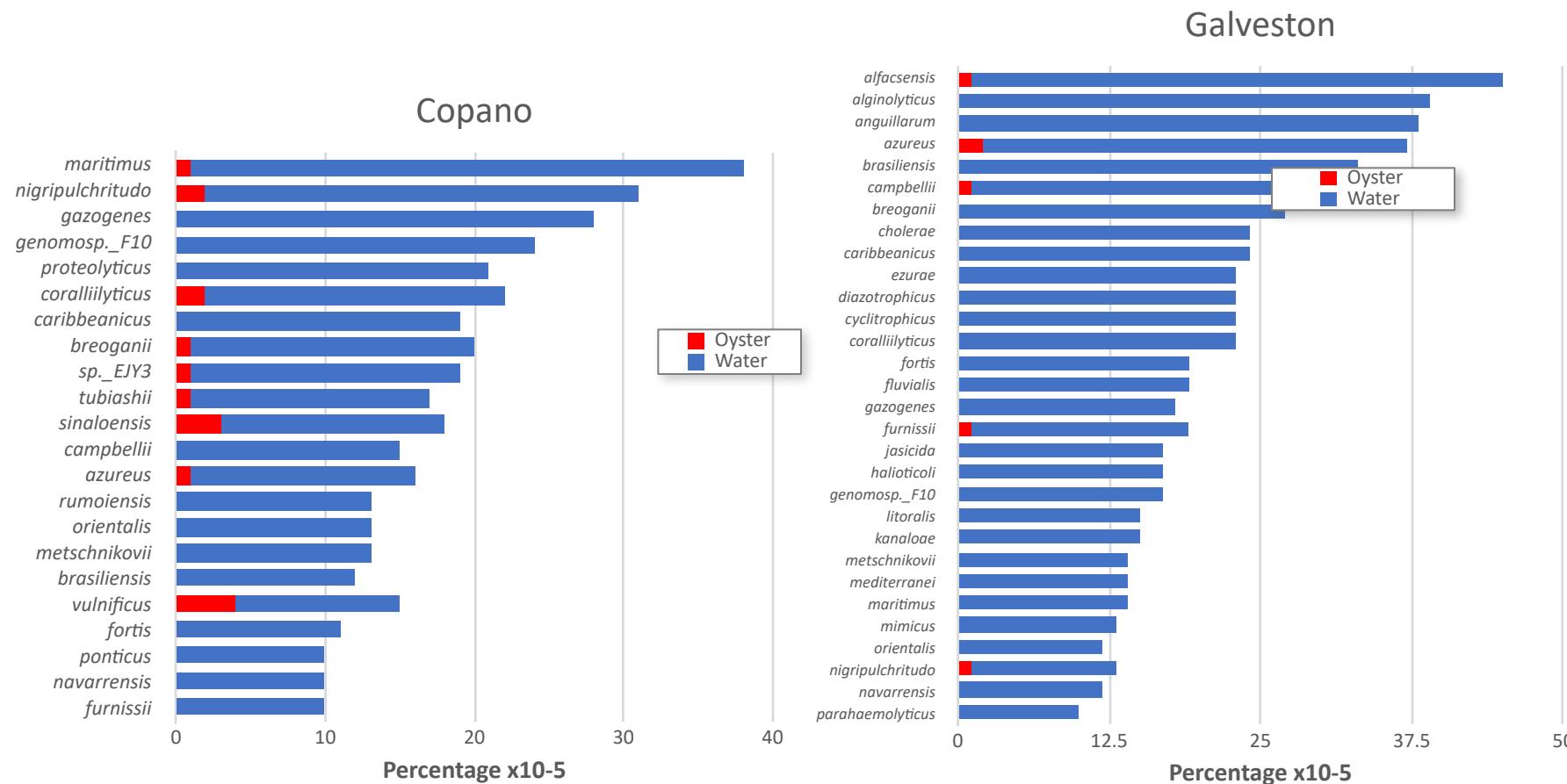
Water (45 OTU)



Oyster (10 OTU)



Vibrio spp. in Texas water and oysters

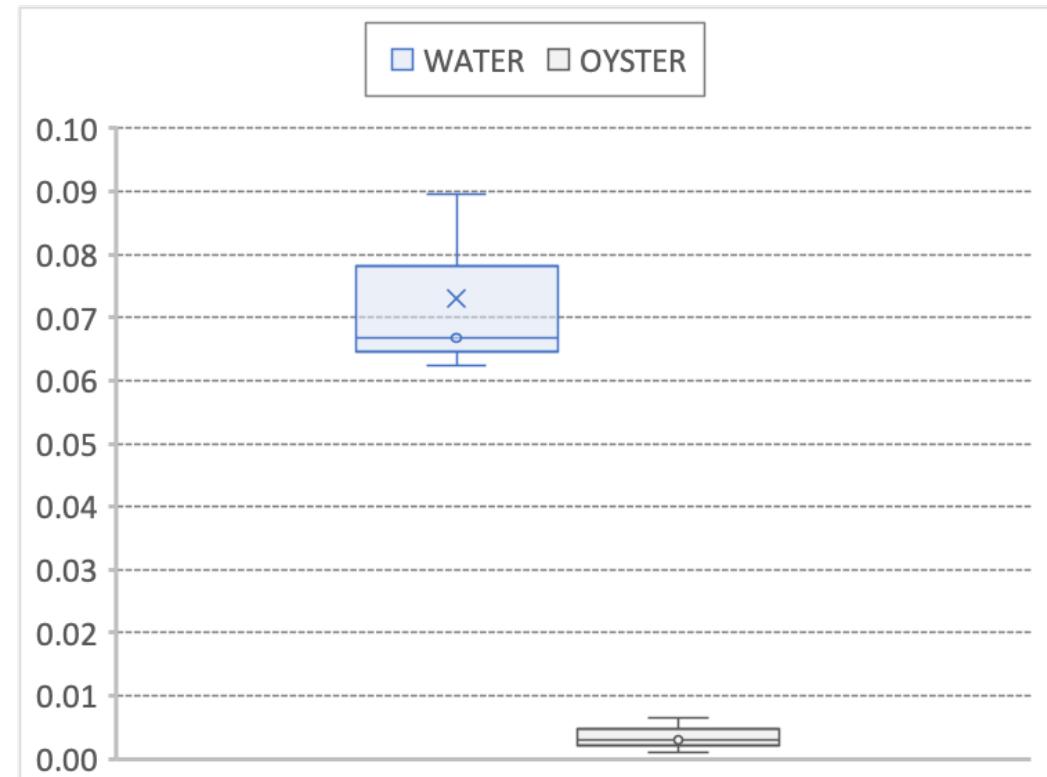


Oyster bacterial community

- High variation between distant localities
- Dominated by few phyla
 - Tenericutes
 - Spirochaetes
 - Proteobacteria
- Own microbiota different from surrounding water

Conclusions (oyster microbiota)

- Vibrios are a tiny fraction of the microbiota of oysters
- Many species found
- Proportion of Vibrios is higher in water than in oysters, about 20X



Metagenómica de Líquido Cefalorraquídeo y Abdominal

Ejemplo del uso de metagenómica para diagnóstico
médico

Antecedentes

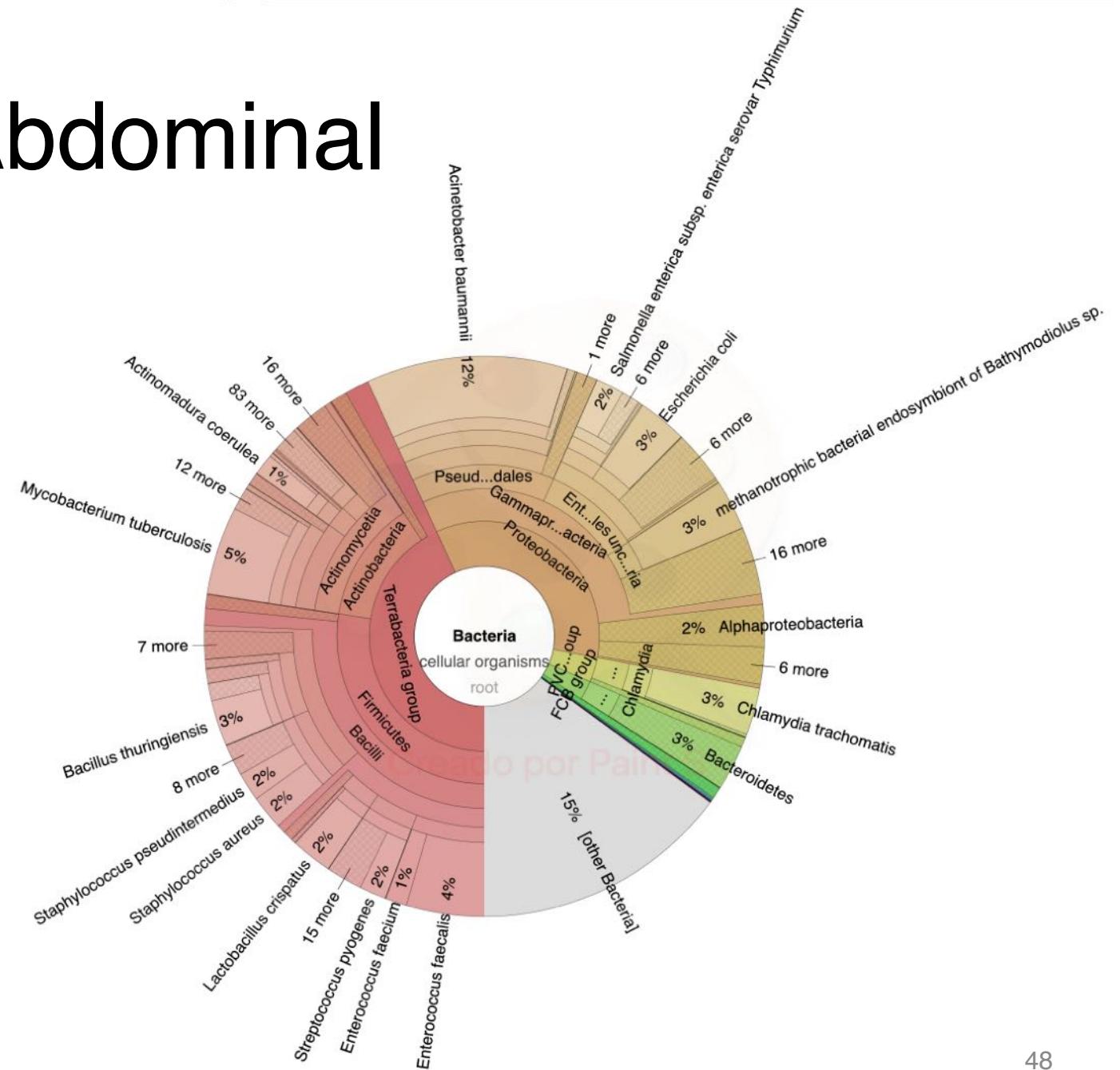
- Paciente femenino
- Internada con hidrocefalia
- Cultivo muy difícil y escaso
- Muestra de Líquido Cefalorraquídeo y Abdominal
- Se extrajo ADN de las muestras y se secuenció en la plataforma Illumina Miniseq
- Análisis bioinformático
 - Creación contigs
 - Clasificación taxonómica y funcional



Fast and sensitive taxonomic classification for metagenomics

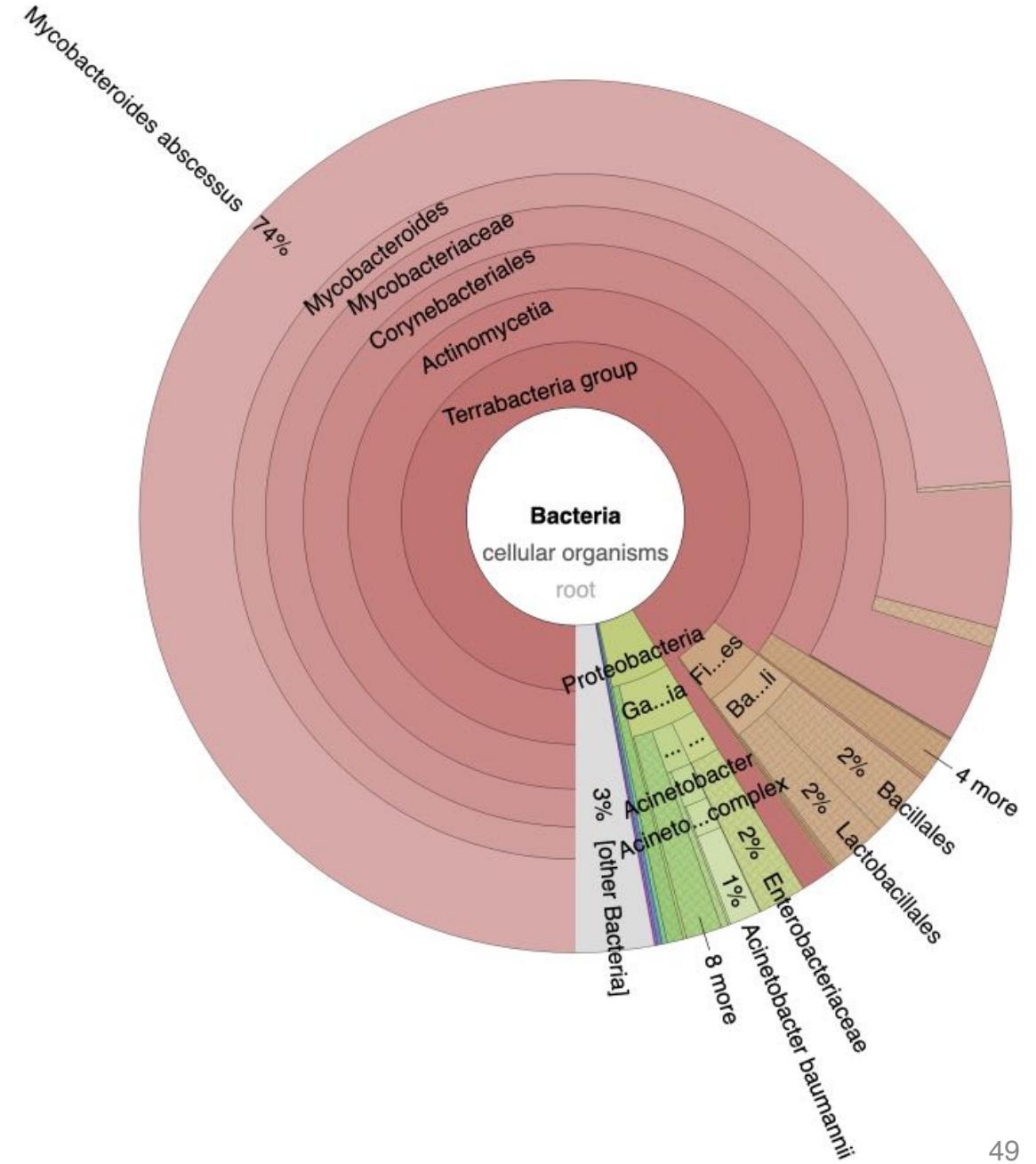
Resultados Líquido Abdominal

- 487,840 secuencias
 - 66.4% clasificadas
 - 2,655 OTUs (taxones)
 - Principales taxones encontrados:
 - *Acinetobacter baumannii*
 - *Mycobacterium tuberculosis*
 - *Enterococcus faecalis*



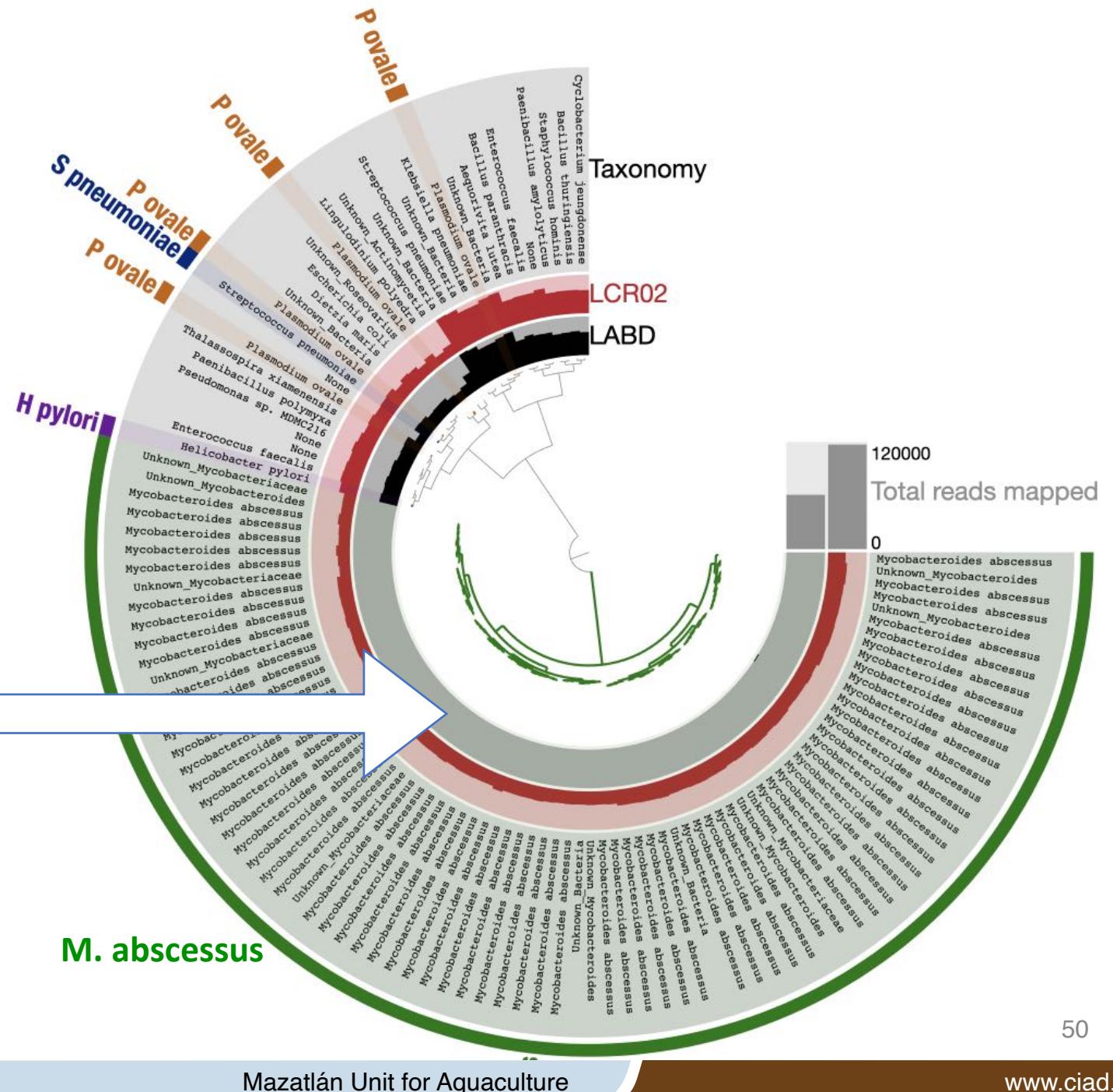
Resultados LCR

- 2.1 millones de secuencias
- 4,599 contigs



Comparación LCR y LAb

M. abscessus ausente en LAb

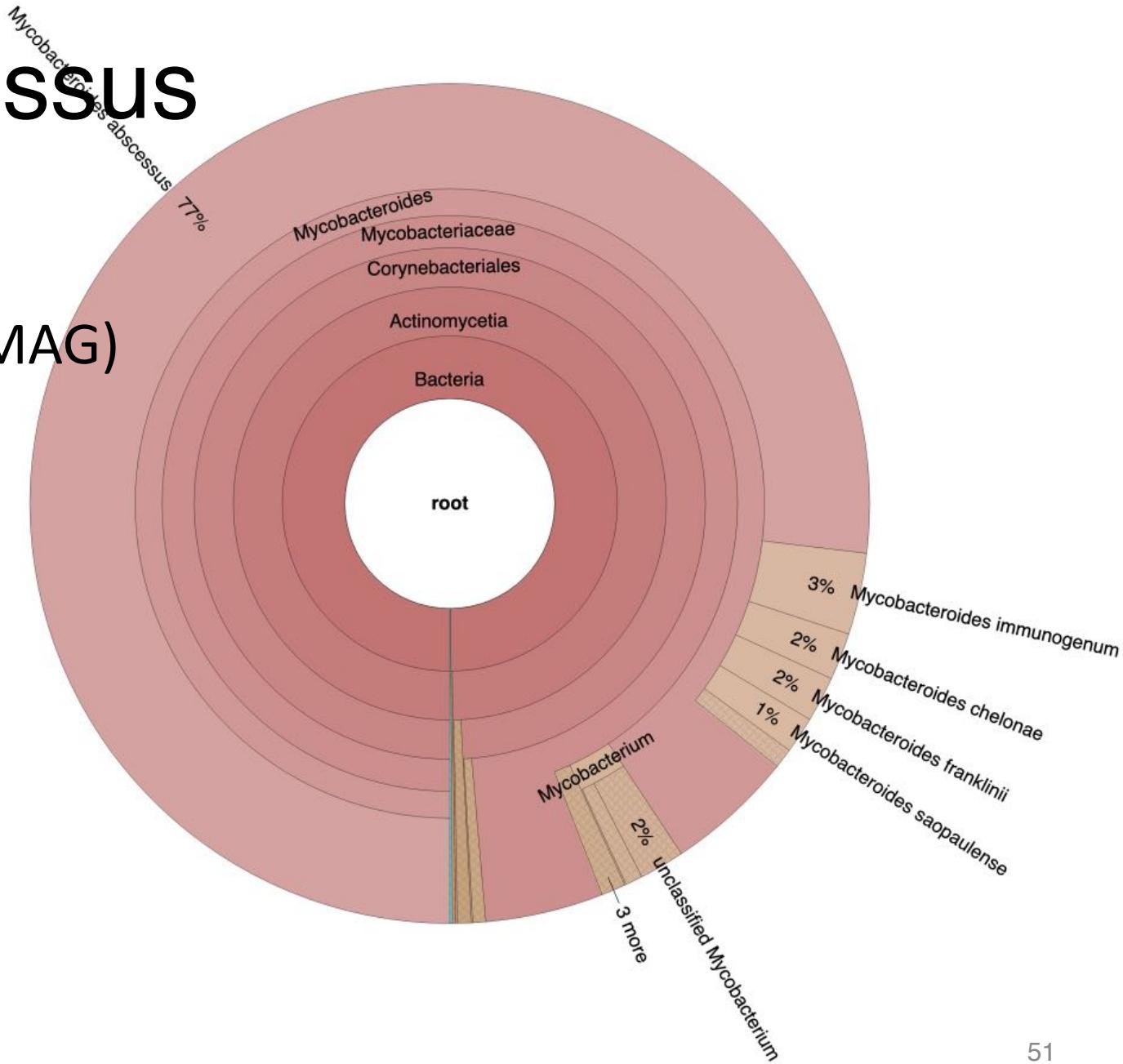


Mycobacterium abscessus

= *Mycobacteroides*

Metagenome Assembled Genome (MAG)

- 1,467 contigs
- 4.35 Mb genome
- 5,471 genes
- 88.7 % completo



Clasificación funcional de *M. abscessus*

Categoría KEGG	Incompleto	Completo	Total general
Amino acid metabolism	23	13	36
Biosynthesis of other secondary metabolites	8		8
Biosynthesis of terpenoids and polyketides	9		9
Carbohydrate metabolism	18	13	31
Energy metabolism	23	4	27
Glycan metabolism	3		3
Lipid metabolism	12	3	15
Metabolism of cofactors and vitamins	32	10	42
Nucleotide metabolism	1	7	8
Xenobiotics biodegradation	3	1	4
Total general	132	51	183



Resistoma en *M. abscessus*

- Genes clasificados con CARD

Tabla 1. Genes clasificados con alta probabilidad a genes de resistencia/

Descripción	Mejor Id.*
23S rRNA with mutation conferring resistance to macrolide antibiotics	
macrolide antibiotic	100.0
RbpA bacterial RNA polymerase-binding protein	
rifamycin antibiotic	87.4
resistance-nodulation-cell division (RND) antibiotic efflux pump	
macrolide antibiotic; penam	95.1

* porcentaje de identidad a un gen conocido en CARD.

Varios otros genes con menor porcentaje de identidad (< 40%)

Gracias

bruno@ciad.mx