Adding biogeochemical meaning to the tree of life

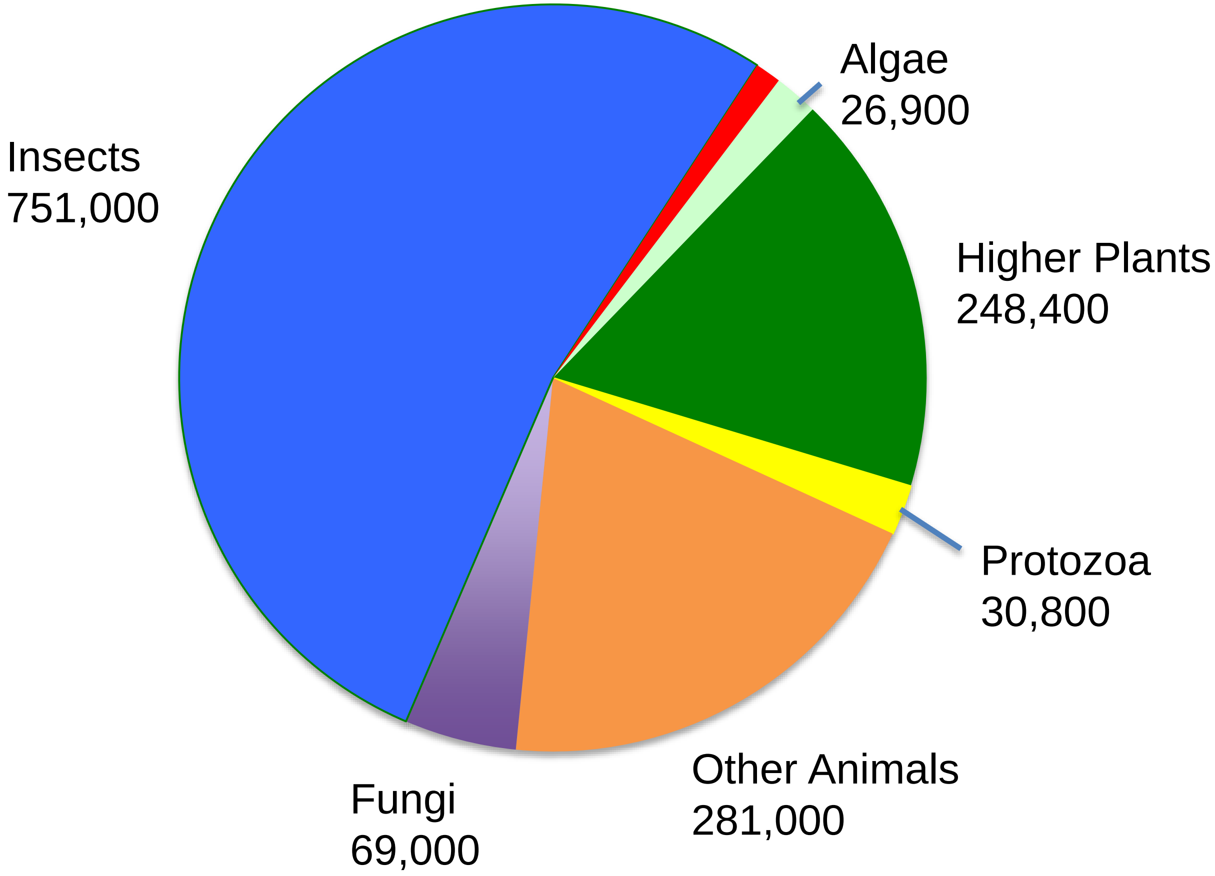
David A. Stahl

April 2018 meeting of BERAC

University of Washington, Seattle
Civil and Environmental Engineering

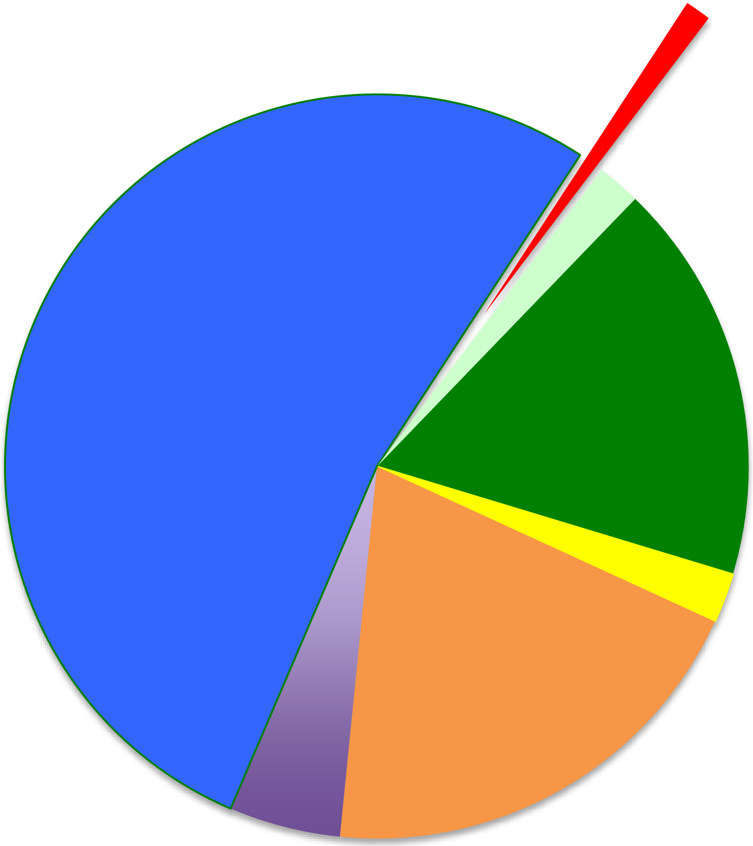
Recognized Diversity of Life

1,423,000 Formally Described Species



Adapted from E.O. Wilson, The Diversity of Life

Recognized Diversity of Life



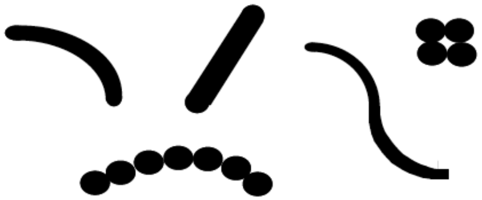
16,054 described species of *Archaea* & *Bacteria* (as of March 2018)

Adapted from E.O. Wilson, The Diversity of Life

Poor representation of microbial diversity in culture collections

Failure to Develop a Natural History of Microorganisms

- They are very small (intimate strangers)
- Direct microscopic observations are not informative



The simple shapes of bacteria conceal
their remarkable diversity

- Culture-based descriptions are highly biased

Most microorganisms fail to grow using conventional cultivation methods

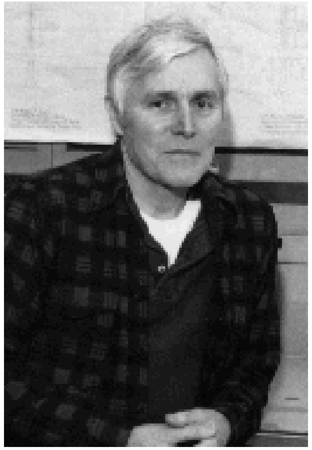
- Physiological descriptions failed to resolve phylogenetic relationships

Development of a phylogenetic framework for microbes

University of Illinois

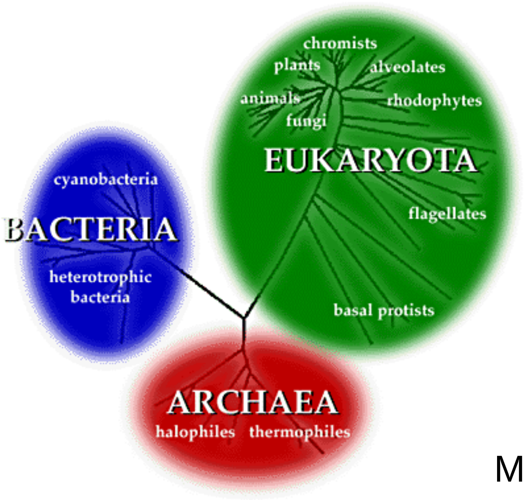
Sol Spiegelman

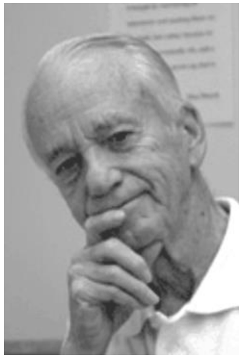
(1914 - 1983)



Carl Woese

(1928 - 2012)





Ralph Wolfe

Methanogen Biochemistry

Developed a method for hybridization of RNA to DNA immobilized on membrane supports. Demonstrated high sequence conservation of the ribosomal RNAs.

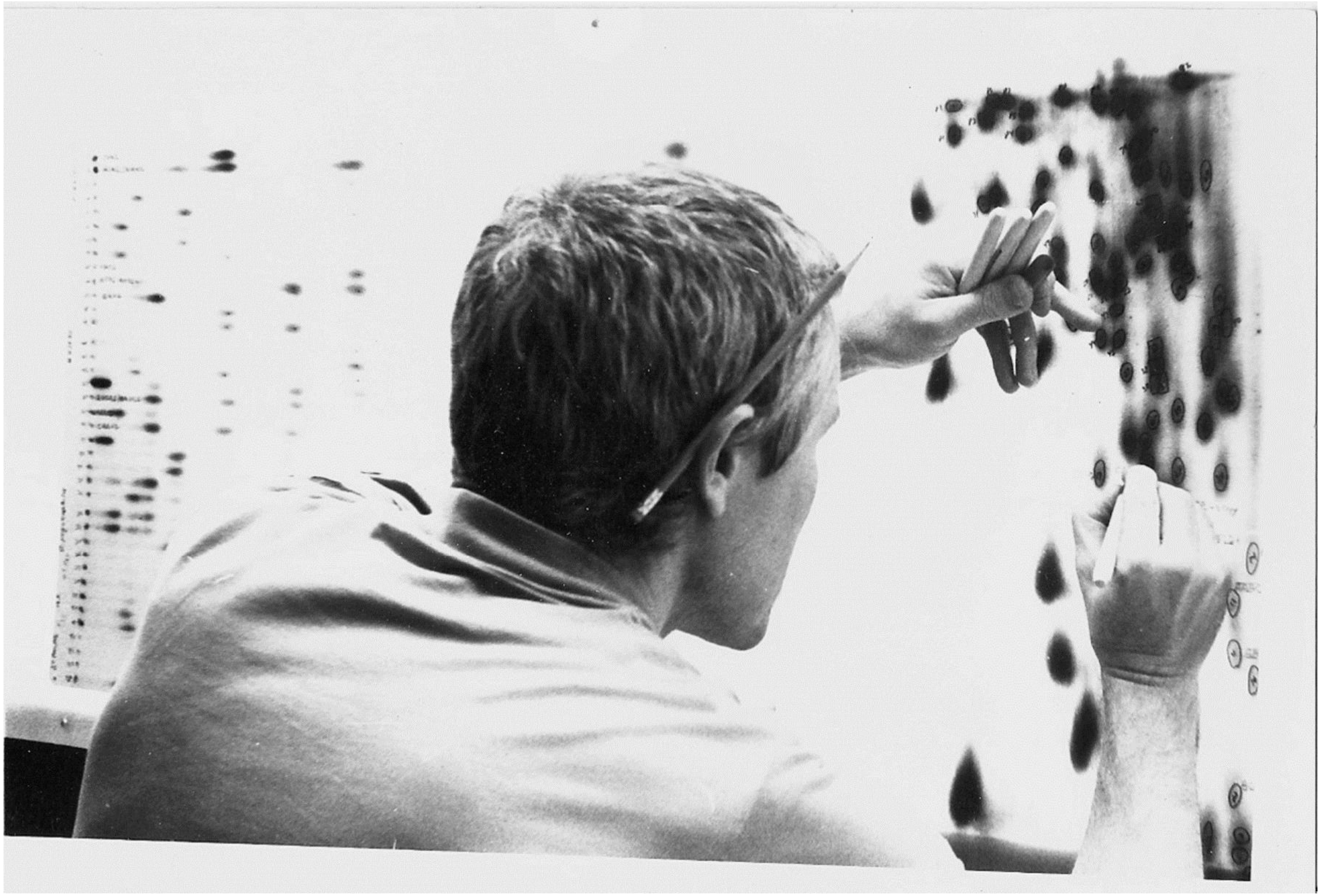
Hall, B.D. & S. Spiegelman. 1961. Sequence complementarity of T2-DNA and T2-specific RNA. Proc. Nat. Acad. Sci. USA 47: 137-46.

"I had set up a program to determine a universal phylogenetic framework, using molecular sequence comparisons (16S rRNAs). At the time, no one really knew what these relationships were, especially among the bacteria. The *Archaea* emerged as the program unfolded."
Woese, The Crafoord Lecture 2003

Fox, G.E. […] Woese, C.R. 1980. The Phylogeny of Prokaryotes. *Science* 209: 457-463

Partial sequencing of the 16S rRNA (sequence catalog)

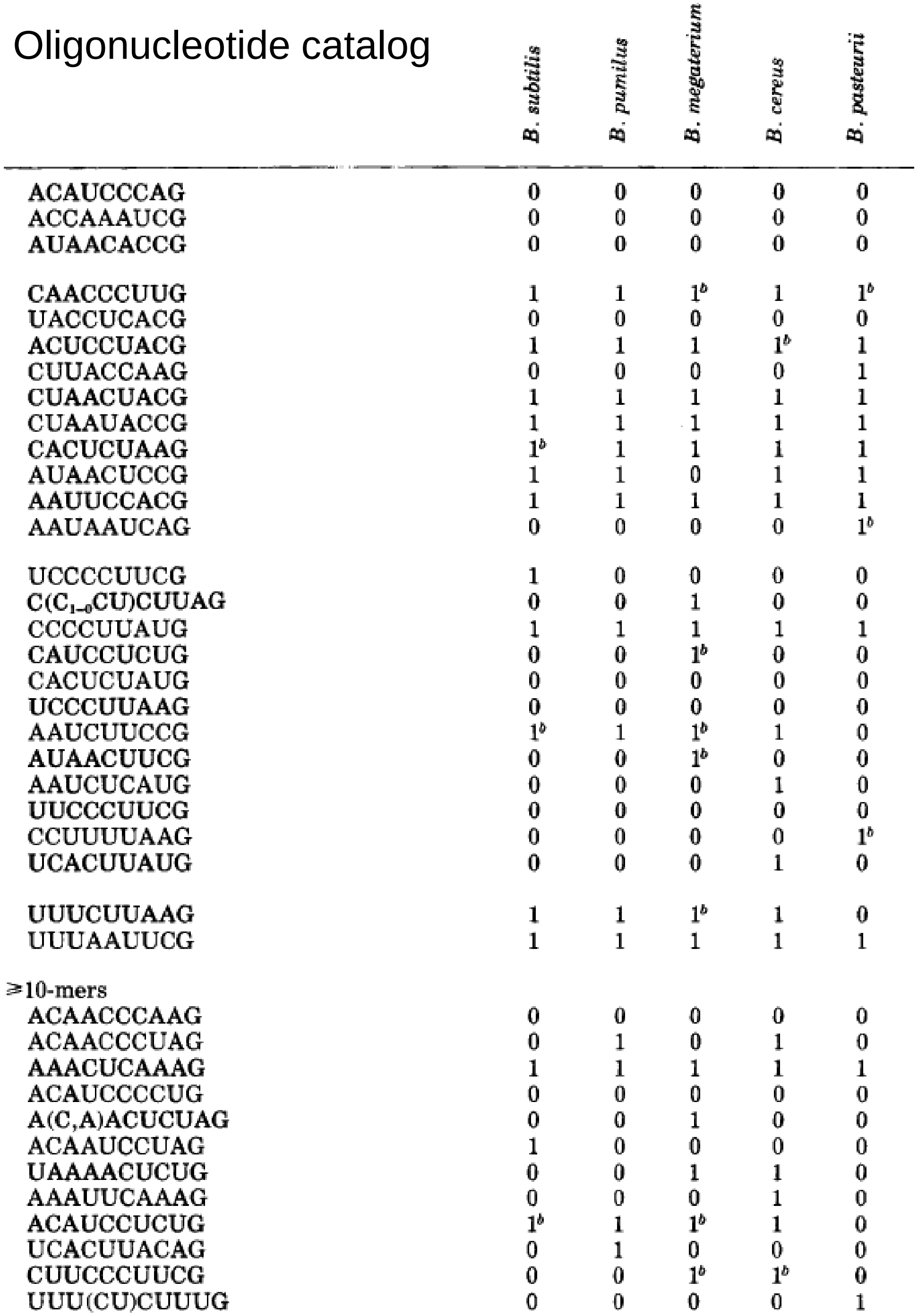
using a “primitive” paper chromatographic method



RNase T_1 Fingerprint

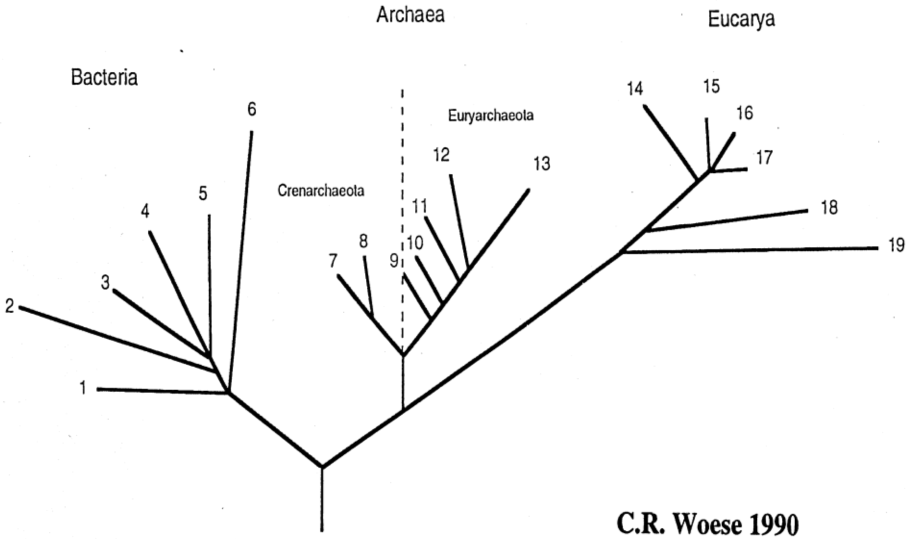
T_1 cleaves after G residues

Oligonucleotide catalog



	B. subtilis	*B. pumilus*	*B. megaterium*	*B. cereus*	*B. pasteurii*
ACAUCCCAG	0	0	0	0	0
ACCAAAUCG	0	0	0	0	0
AUAACACCG	0	0	0	0	0
CAACCCUUG	1	1	1^b	1	1^b
UACCUCACG	0	0	0	0	0
ACUCCUACG	1	1	1	1^b	1
CUUACCAAG	0	0	0	0	1
CUAACUACG	1	1	1	1	1
CUAAUACCG	1	1	1	1	1
CACUCUAAG	1^b	1	1	1	1
AUAACUCCG	1	1	0	1	1
AAUUCCACG	1	1	1	1	1
AAUAAUCAG	0	0	0	0	1^b
UCCCCUUCG	1	0	0	0	0
C(C$_{1-6}$CU)CUUAG	0	0	1	0	0
CCCCUUAUG	1	1	1	1	1
CAUCCUCUG	0	0	1^b	0	0
CACUCUAUG	0	0	0	0	0
UCCCUUAAG	0	0	0	0	0
AAUCUUCCG	1^b	1	1^b	1	0
AUAACUUCG	0	0	1^b	0	0
AAUCUCAUG	0	0	0	1	0
UUCCCUUCG	0	0	0	0	0
CCUUUUAAG	0	0	0	0	1^b
UCACUUAUG	0	0	0	1	0
UUUCUUAAG	1	1	1^b	1	0
UUUAAUUCG	1	1	1	1	1
⩾10-mers					
ACAACCCAAG	0	0	0	0	0
ACAACCCUAG	0	1	0	1	0
AAACUCAAAG	1	1	1	1	1
ACAUCCCCUG	0	0	0	0	0
A(C,A)ACUCUAG	0	0	1	0	0
ACAAUCCUAG	1	0	0	0	0
UAAAACUCUG	0	0	1	1	0
AAAUUCAAAG	0	0	0	1	0
ACAUCCUCUG	1^b	1	1^b	1	0
UCACUUACAG	0	1	0	0	0
CUUCCCUUCG	0	0	1^b	1^b	0
UUU(CU)CUUUG	0	0	0	0	1

The Foundation for a Natural History of Microorganisms

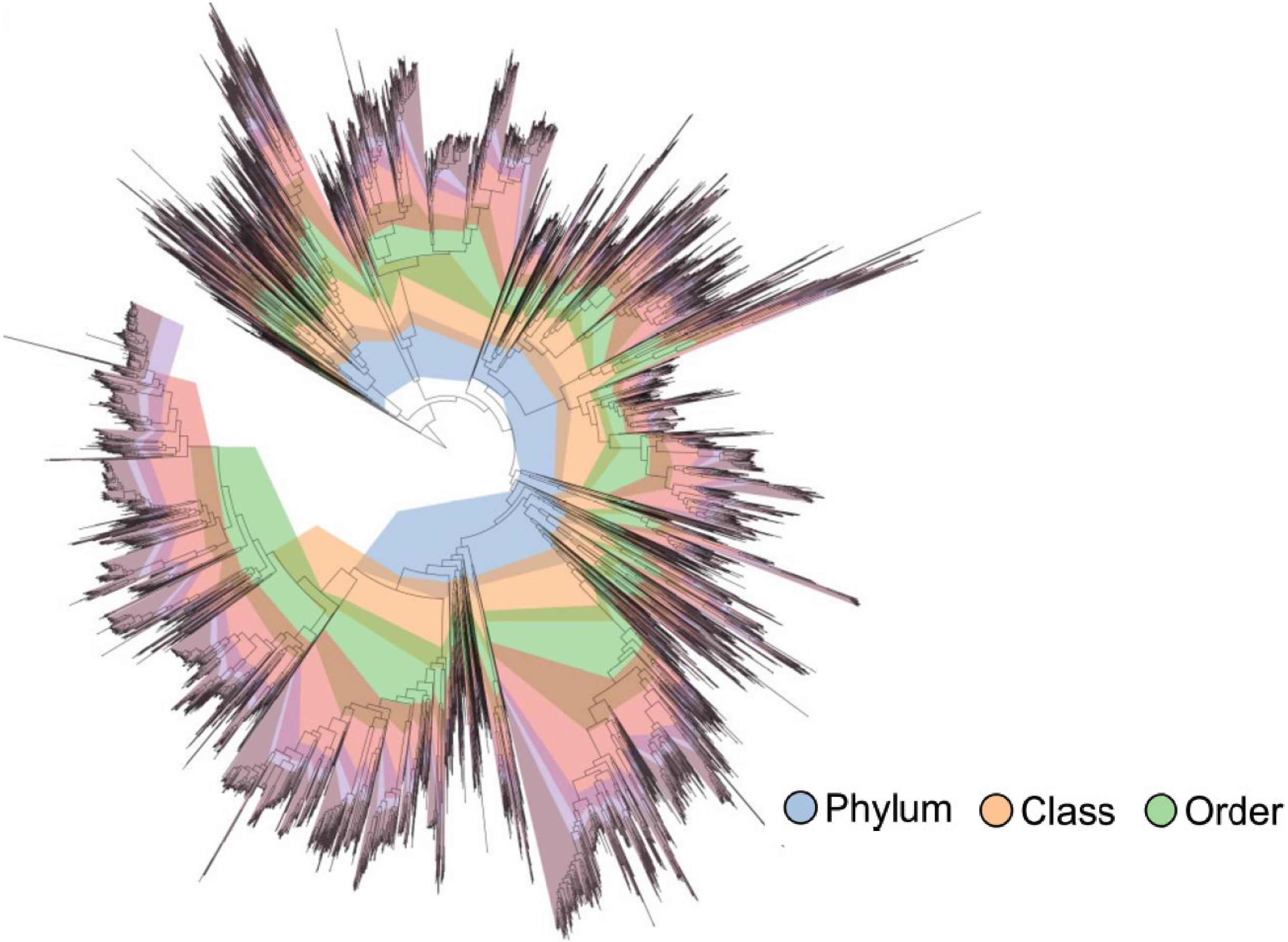


C.R. Woese 1990

Gene sequences recovered directly from environmental samples could now be related to known (cultured) organisms and other yet-to-be cultured organisms. Six bacterial “phyla” initially described.

Most recent census of diversity within the bacterial domain

99 phyla recently described, based on 95,000 bacterial (meta)genomes



Parks, Chuvochina, Waite, Rinke, Skarshewski, Chaumeil, Hugenholtz. BioRxiv 2018

Status

Microbial Species Diversity?

- Still counting – global species estimates range from millions to trillions…

  So… 0.1 - 0.0000001% of microbial species diversity is now formally described

Consequences?

- Very sparse understanding of relationships between microbial diversity, physiology and biogeochemistry

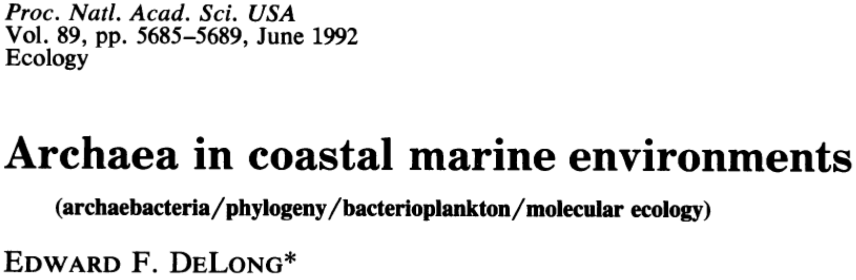
In pursuit of the organisms

Stepping back 26 years

LETTERS
Nature 356, 148-149, March 1992

Novel major archaebacterial group from marine plankton

Jed A. Fuhrman, Kirk McCallum* & Alison A. Davis

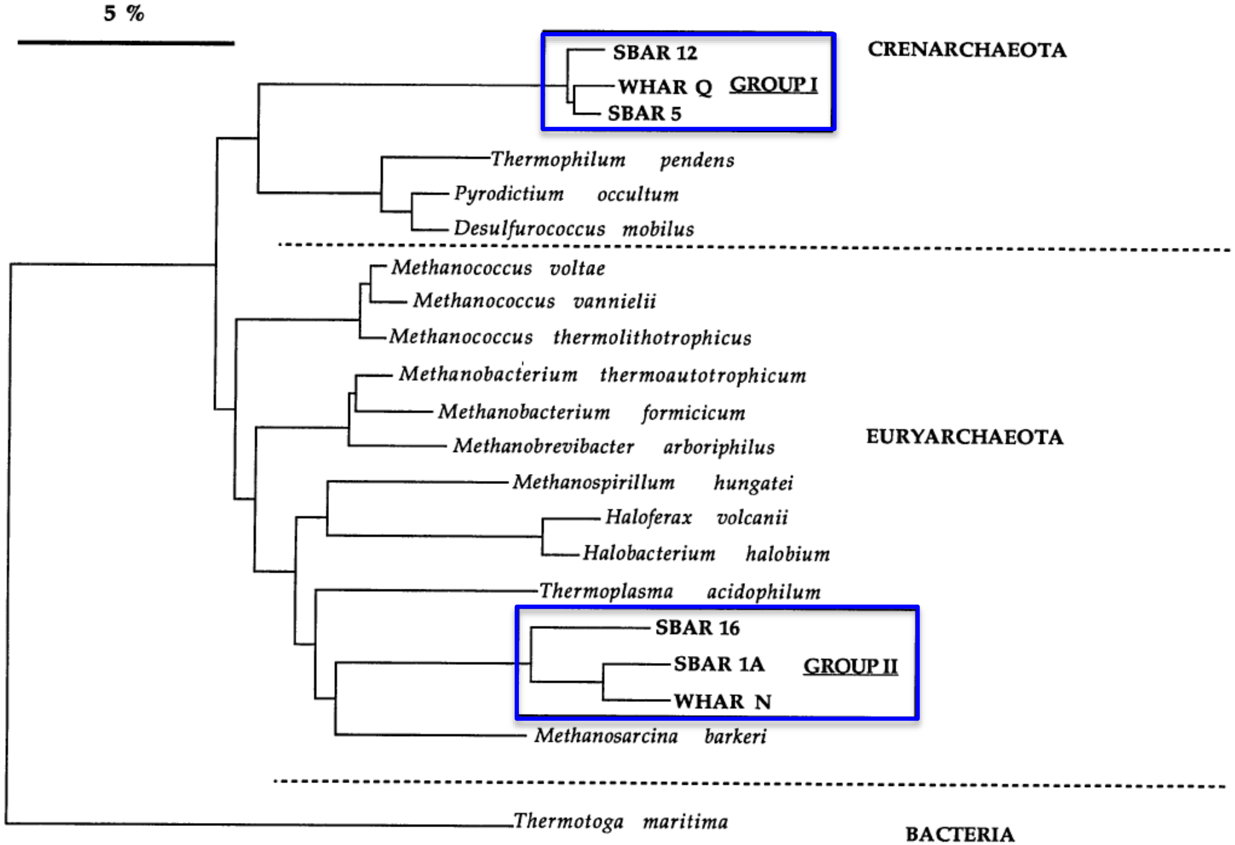


Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5685–5689, June 1992
Ecology

Archaea in coastal marine environments

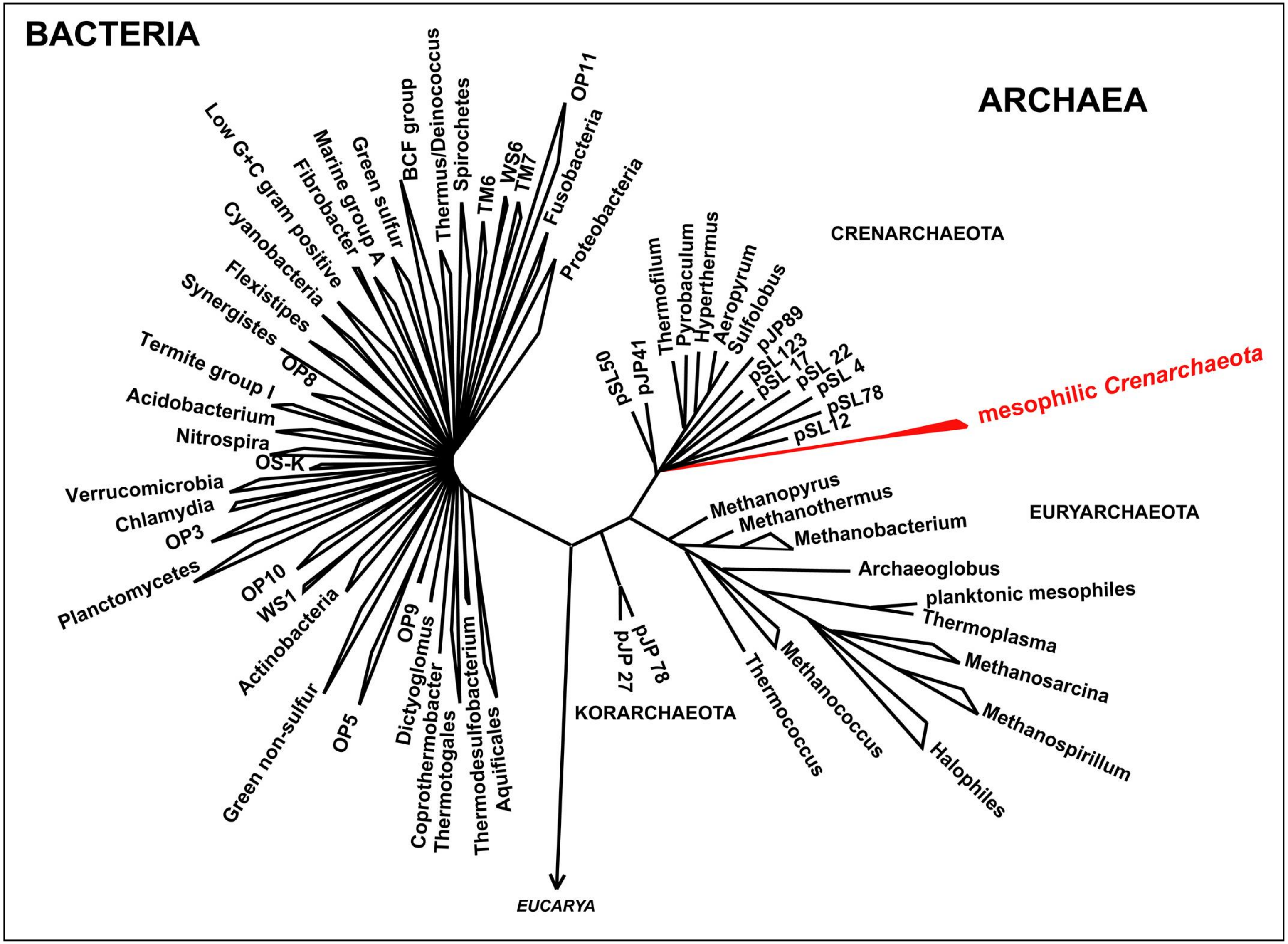
(archaebacteria/phylogeny/bacterioplankton/molecular ecology)

EDWARD F. DELONG*



Mesophilic Group I “*Crenarchaeota*”

Cold (4˚C) oxic seawater (16S rRNA gene PCR)



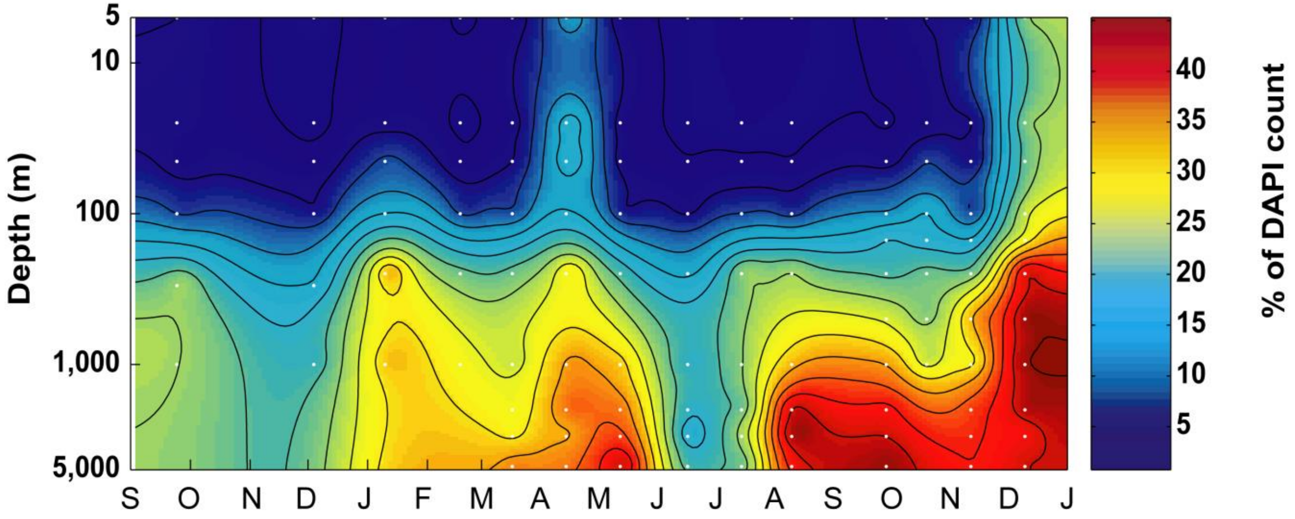
Marine Group 1 "*Crenarchaeota*"

- Account for nearly 20% of all oceanic bacterioplankton (~10^{28} cells), of presumptive biogeochemical significance [*Karner et al., 2001*]

- Isotopic analyses and tracer experiments suggested autotrophy [*Pearson et al., 2001; Wuchter et al. 2003*]

- No cultivated representatives for 23 years following discovery

Abundance of Marine Crenarchaeota at the Hawaii Ocean Time Series (HOTS)



Karner, DeLong & Karl (2001), Nature 409:507-10

Plum Island Sound Estuarine System

NSF Long Term Ecological Research Site

NSF Microbial Observatory

Controls of nitrification in this estuary

With focus on ammonia oxidizing bacteria (AOB)

Sergei Nikolaievich Winogradsky

Nitrification thought solely a bacterial process for over a century

1856-1953

Published in 1890 his research on nitrifying bacteria established the principal of chemolithoautotrophy, the use an inorganic electron donor (here, either ammonia or nitrite) for energy and the reduction of CO_2 to organic carbon.

I. Ammonia-Oxidizing Bacteria (e.g., *Nitrosomonas*, *Nitrosococcus*)

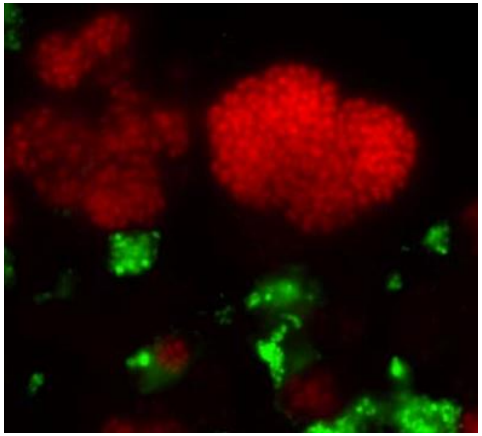
$$NH_4^+ + 2O_2 + NADH \longrightarrow NO_2^- + 2H_2O + NAD^+ + H^+$$

II. Nitrite-Oxidizing Bacteria (e.g., *Nitrobacter*, *Nitrococcus*)

$$NO_2^- + {}^1/_2O_2 \longrightarrow NO_3^-$$

III. Comammox (some *Nitrospira* spp.)

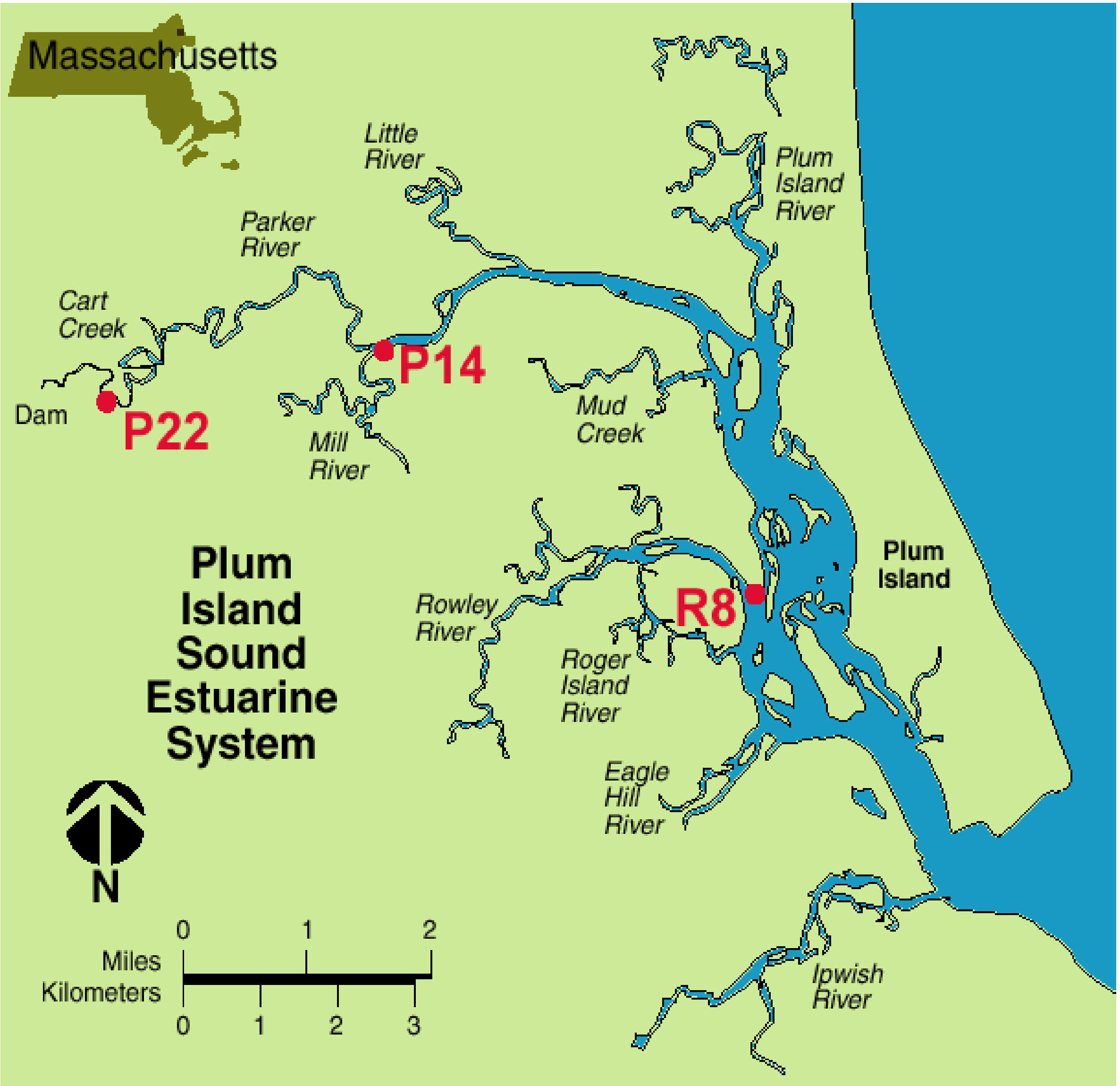
$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + H_2O + 2H^+$$



Daims et al./van Kessel et al. 2015. Nature

Winogradsky, S. 1890. Recherches sur les organismes de la nitrification. Annales de l'Institut Pasteur, 4, 257-275.

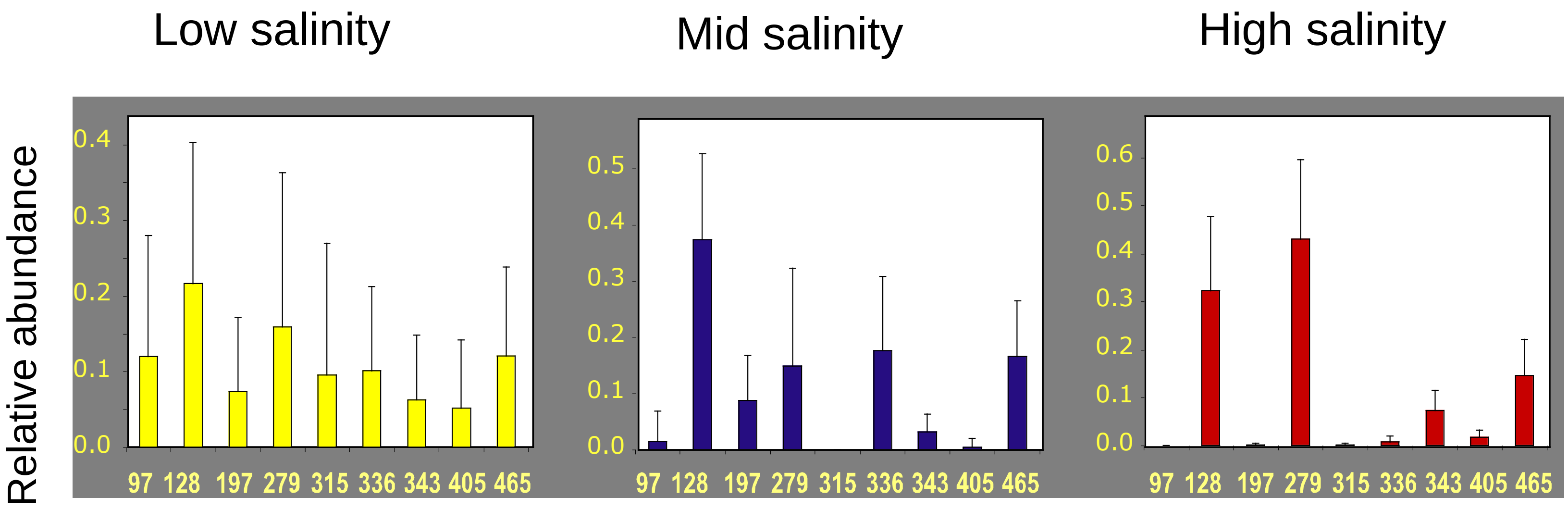
Study sites of varying salinity



Sample collection - Plum Island Spartina tidal flats

Ammonia-oxidizing bacteria (AOB) diversity decreased with increasing salinity

As evaluated by bacterial ammonia monooxygenase gene (*amoA*)

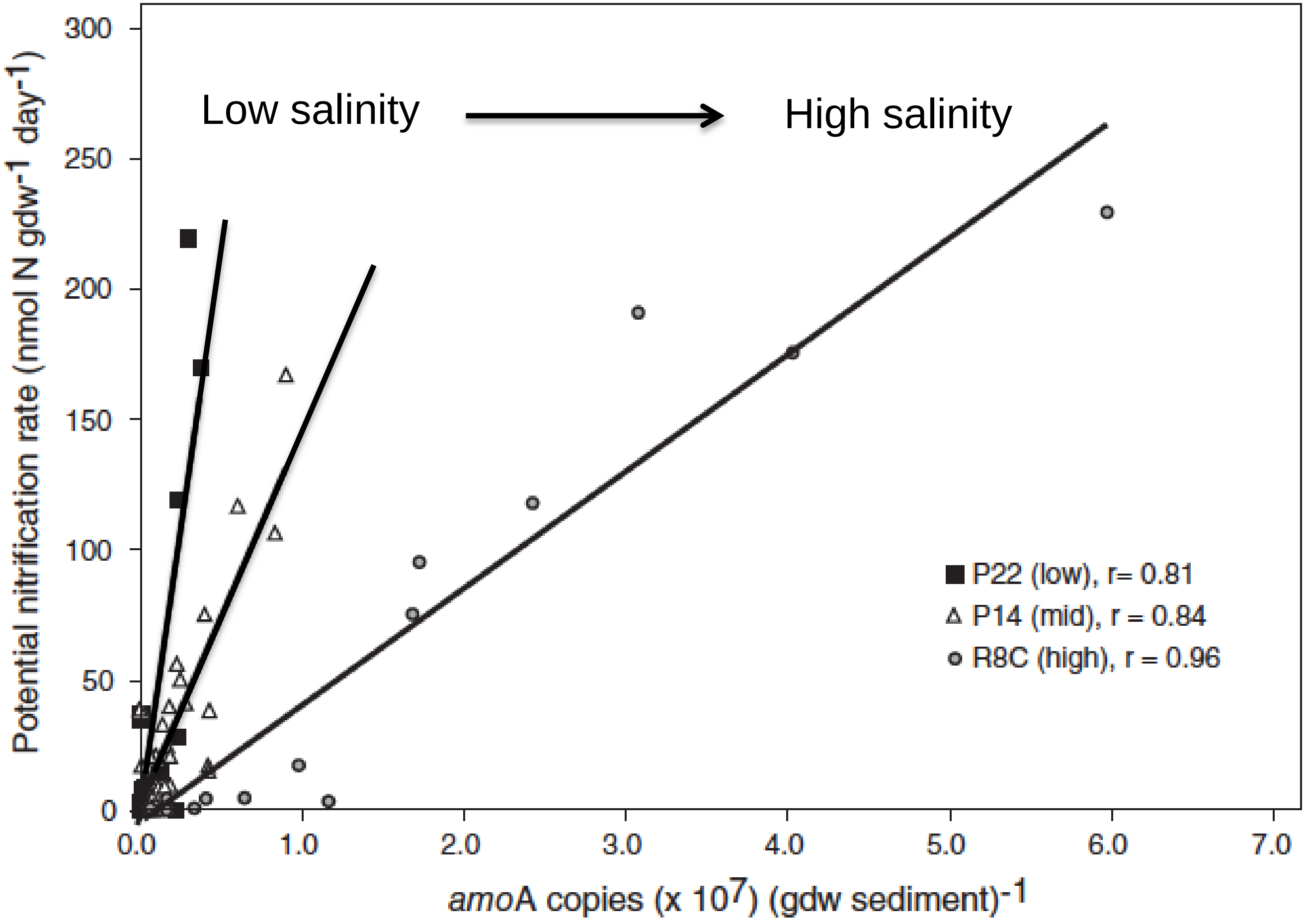


Reduced bacterial *amoA* amplicon diversity →

Bernhard et al. 2005. Environ. Microbiol.

With increasing salinity

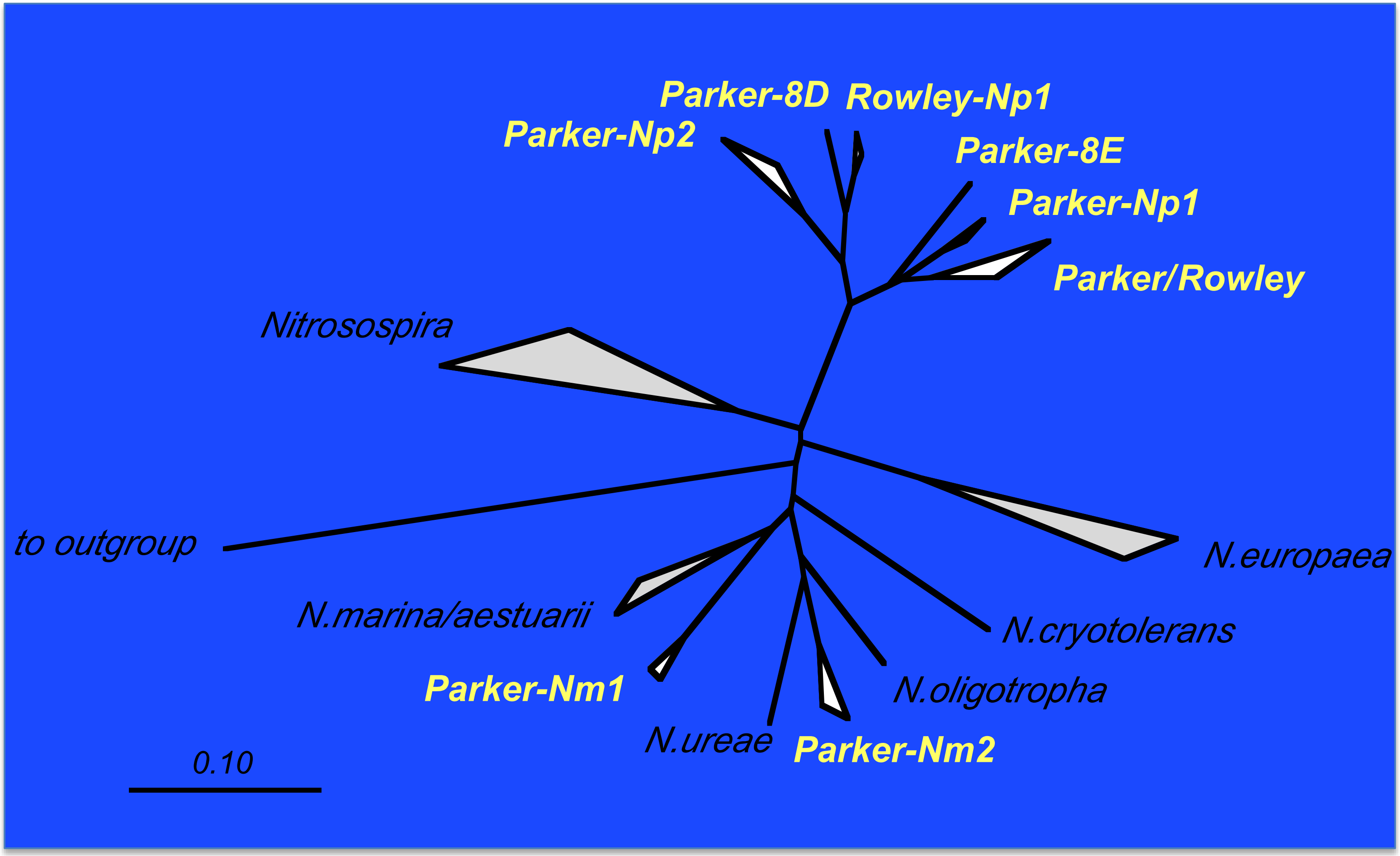
ammonia oxidation rate per bacterial *amoA* gene decreased



Bernhard et al.

Most AOB in Plum Island not closely related to cultivated AOB

as inferred from phylogeny of *amoA*



Enrichment cultures of representative ammonia oxidizers

Enlisted the assistance of John Waterbury and Freddy Valois

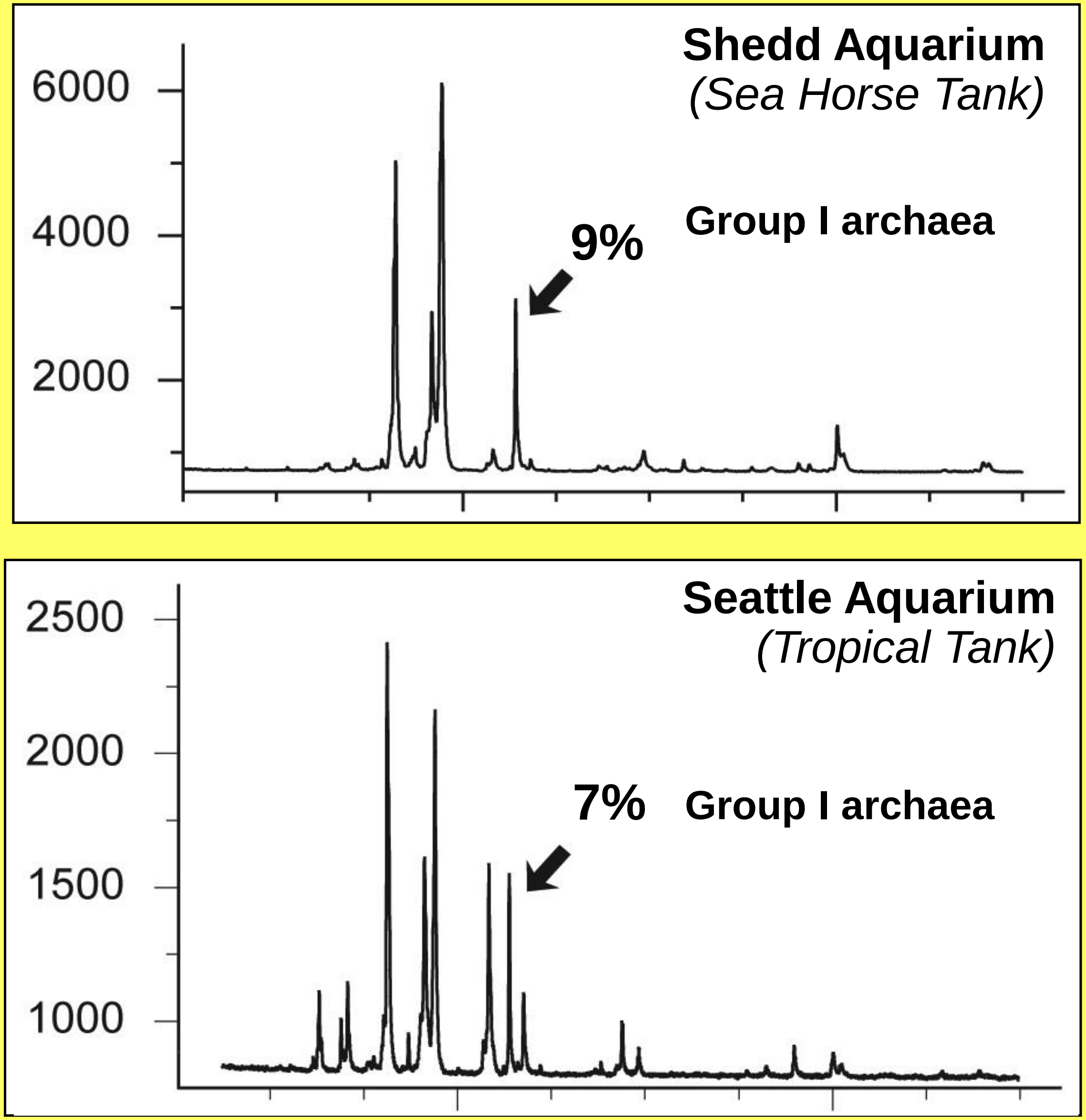
Woods Hole Oceanographic Institution

Absence of the bacterial *amoA* in actively nitrifying enrichments

Similar observations made earlier at the Shedd Aquarium

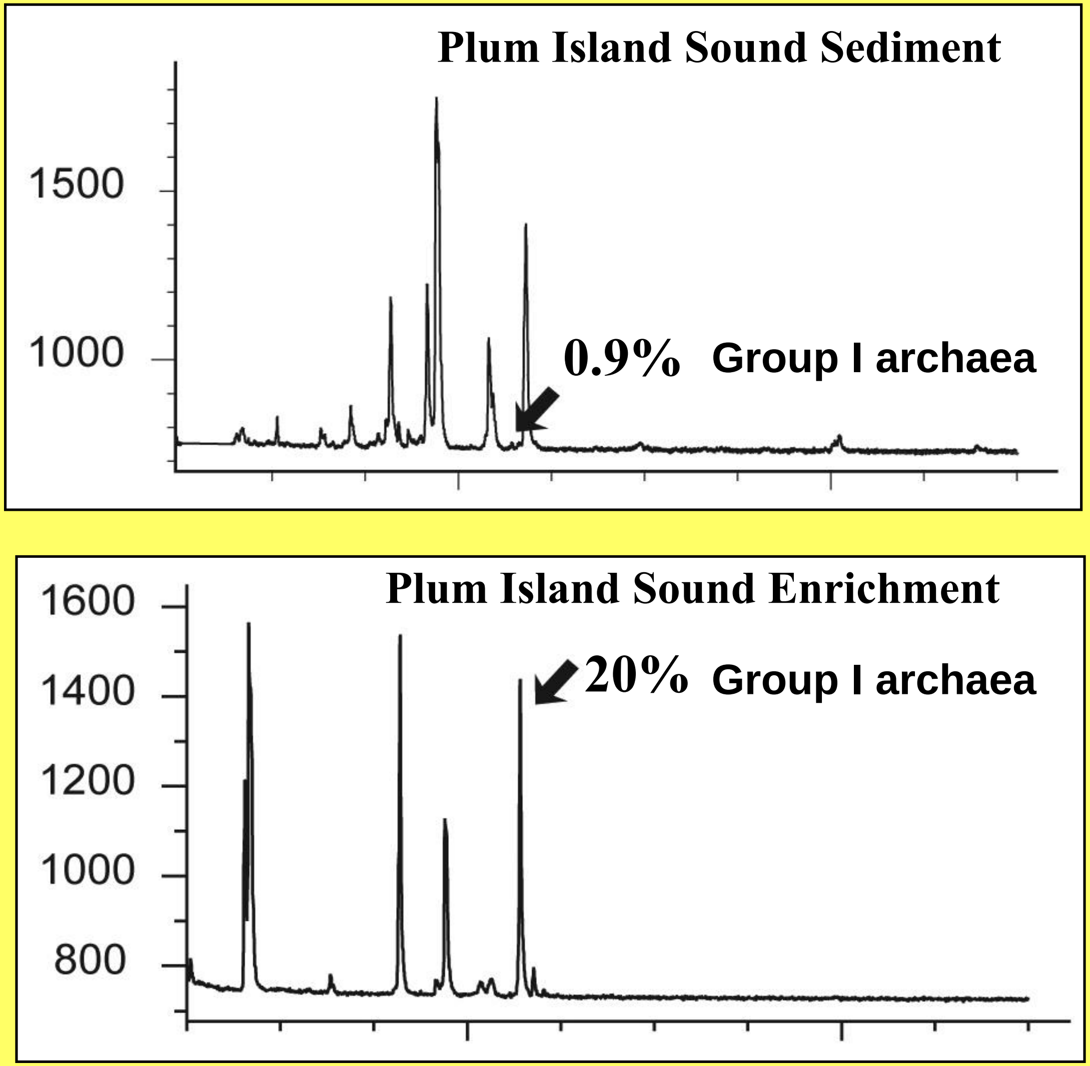
Absence of AOB in active nitrifying marine biofiltration systems

Group I archaea abundant in aquaria nitrifying reactors



Universal 16S rRNA gene T-RFLP Analysis

Highly enriched in nitrifying enrichment cultures from Plumb Island



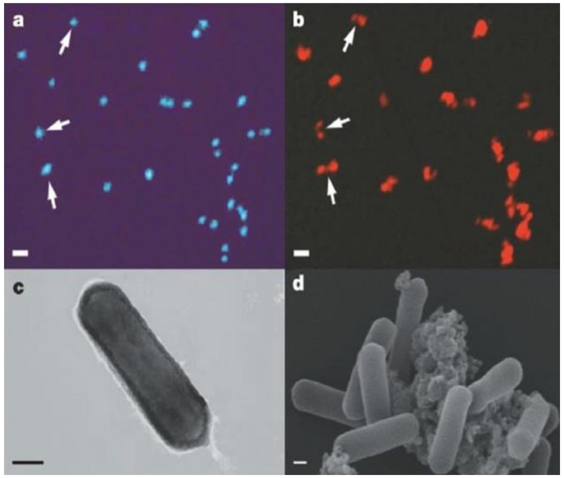
Universal 16S rRNA gene T-RFLP Analysis

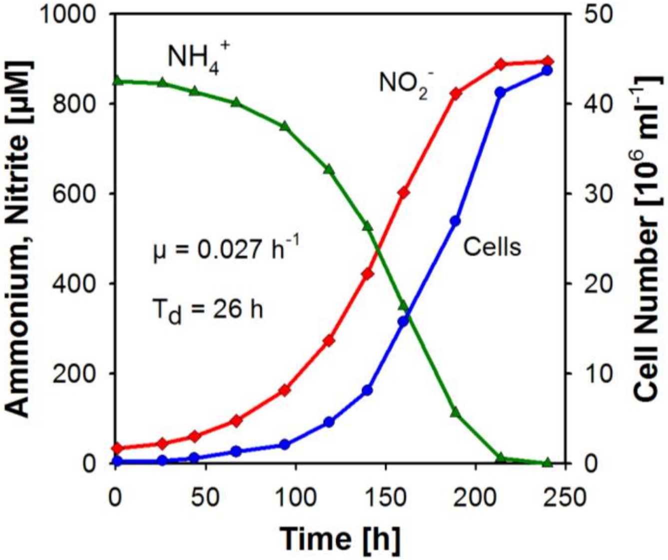
Contribution of Archaea to ammonia oxidation

Established in 2005 with isolation of the first ammonia-oxidizing archaeon (AOA)

Nitrosopumilus maritimus (dwarf nitrifier of the sea)

- Isolated from tropical tank (24° C) at the Seattle Aquarium
- Closely related to *Archaea* abundant in the marine and terrestrial environments
- Chemoautotrophic growth by oxidation of ammonia to nitrite





Koenneke, Bernhard, de la Torre, et al. *Nature* 2005
Martens-Habbena et al., *Nature* 2009

Geothermal Microbiology: Field work in Yellowstone National Park

Heart Lake 1

70-80° C

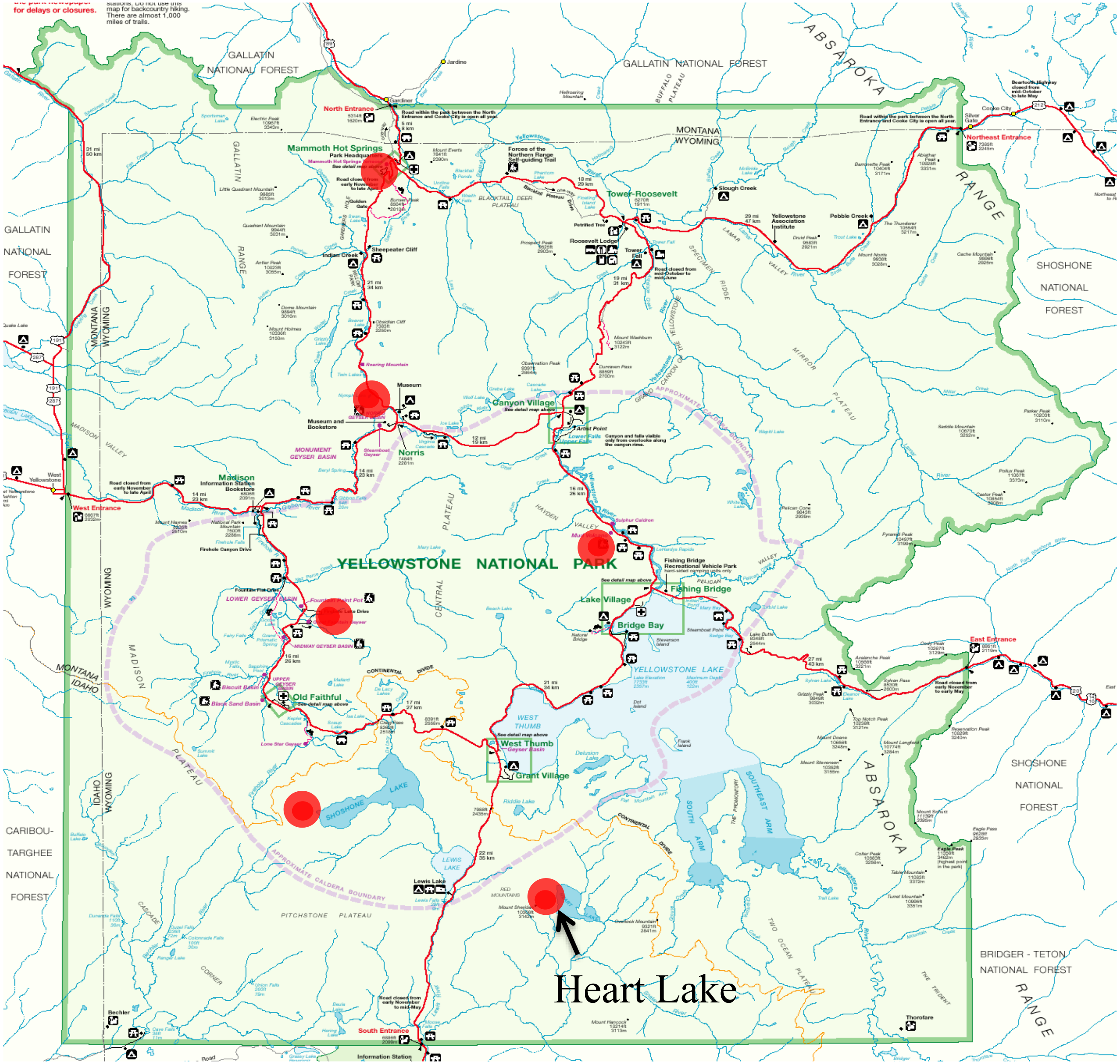
pH 8.3

NH_4^+ 95 µM

NO_2^- 3 µM

NO_3^- 174 µM

Archaeal *amoA* widely distributed in the Park



Heart Lake

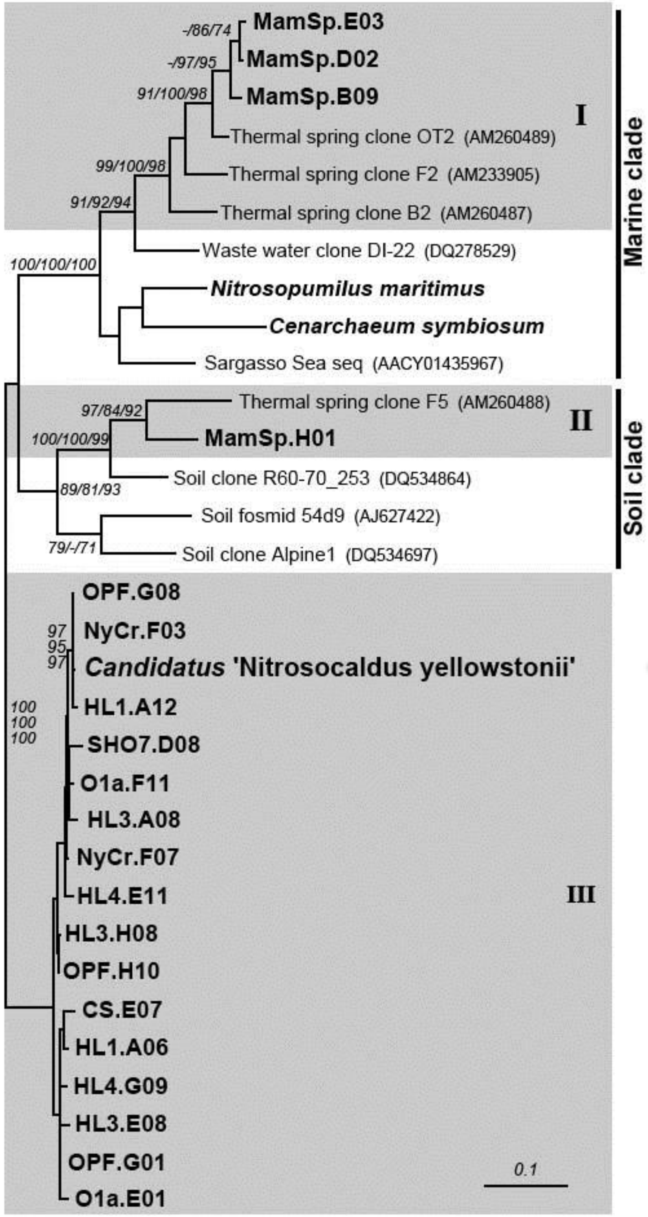
Nymph Creek

pH ~ 3.0

30^{o} C   10 uM NH_4^+

Nitrification in geothermal systems

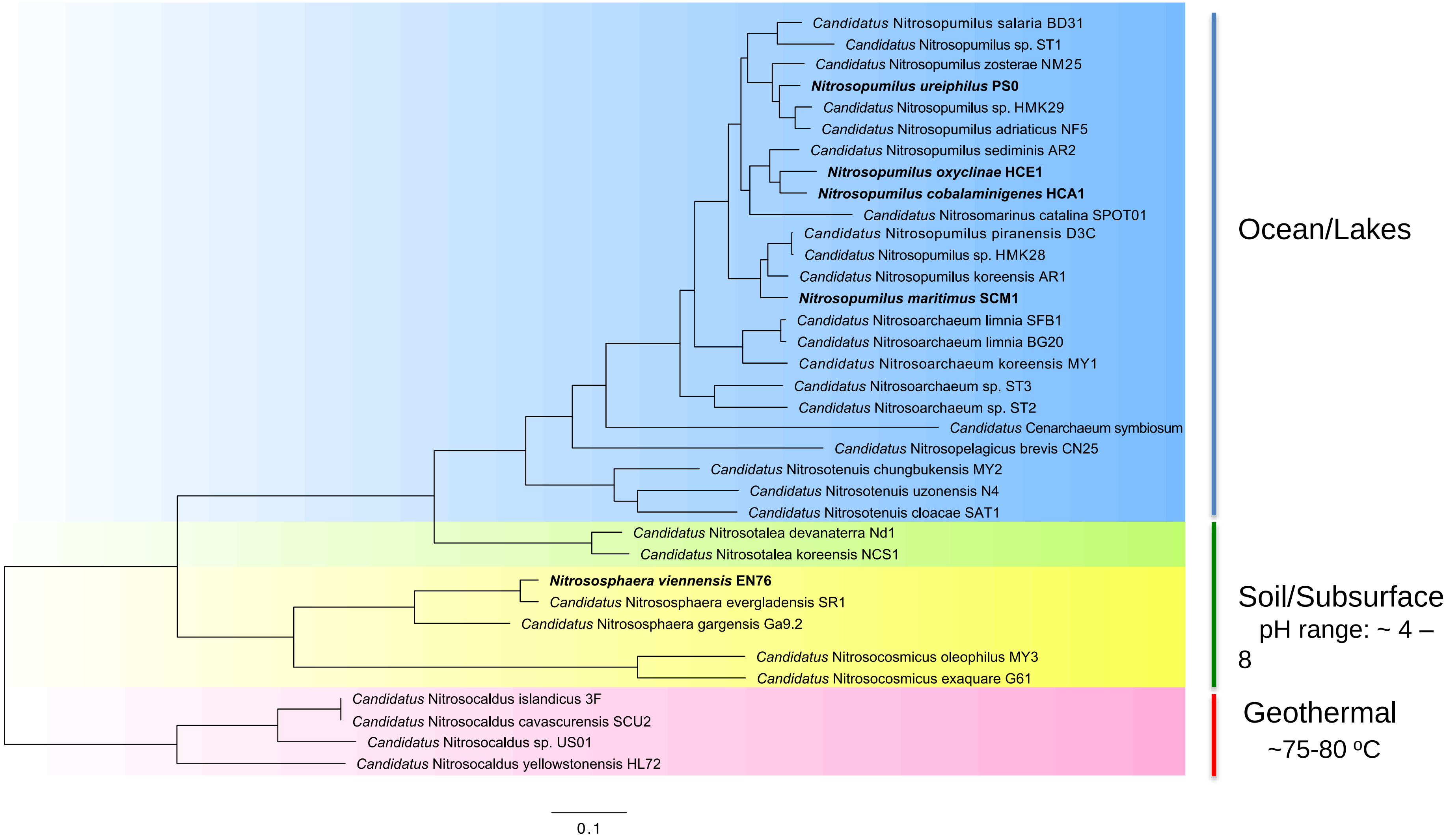
Novel clade of archaeal *amoA* genes widely distributed in geothermal springs



In culture (~ 75° C)

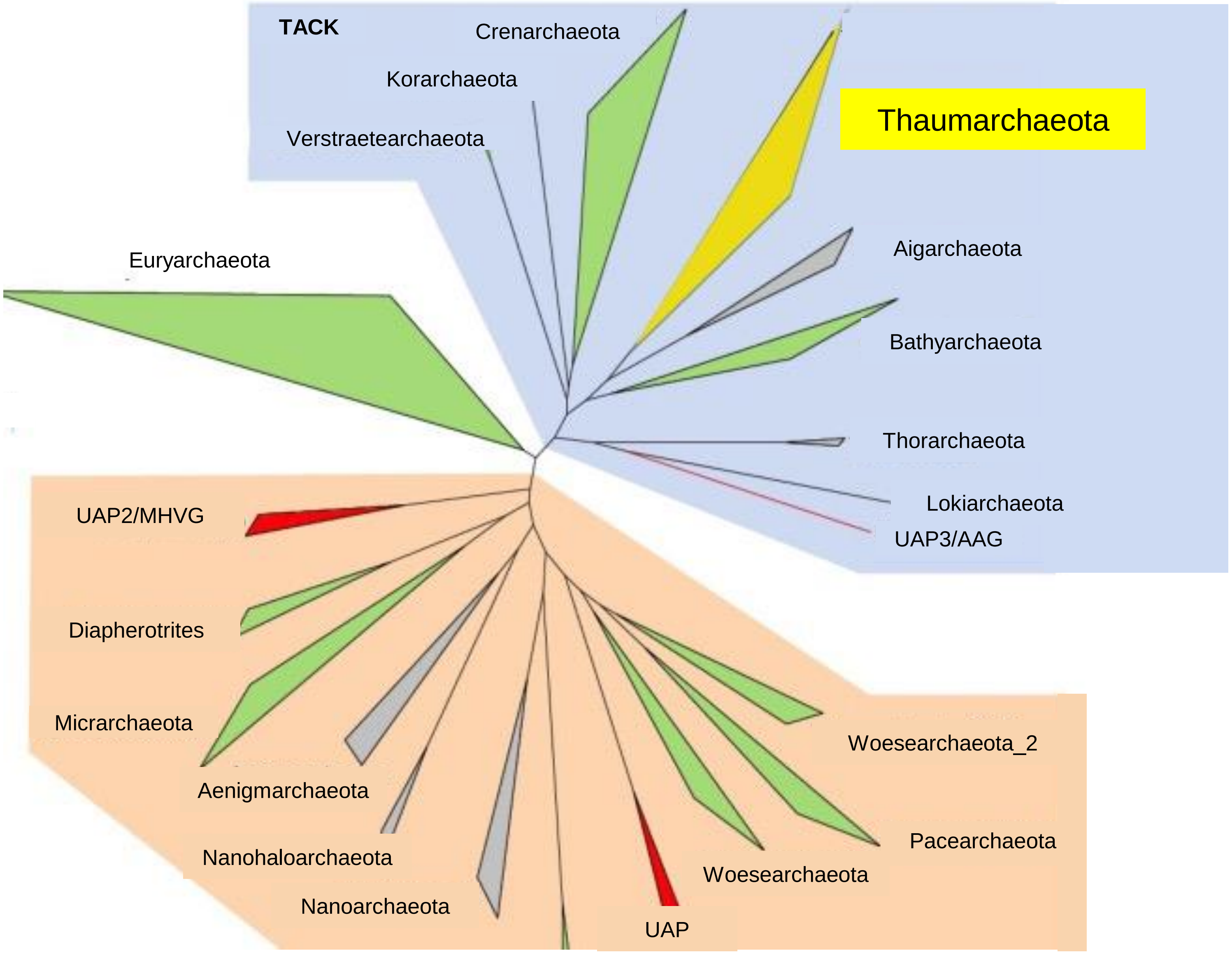
De la Torre et al. 2008. Environ. Microbiol.

Current appreciation of AOA species diversity & habitat range



Qin et al. unpublished

Archaeal ammonia oxidizers solely comprise the recently described *Thaumarchaeota* phylum



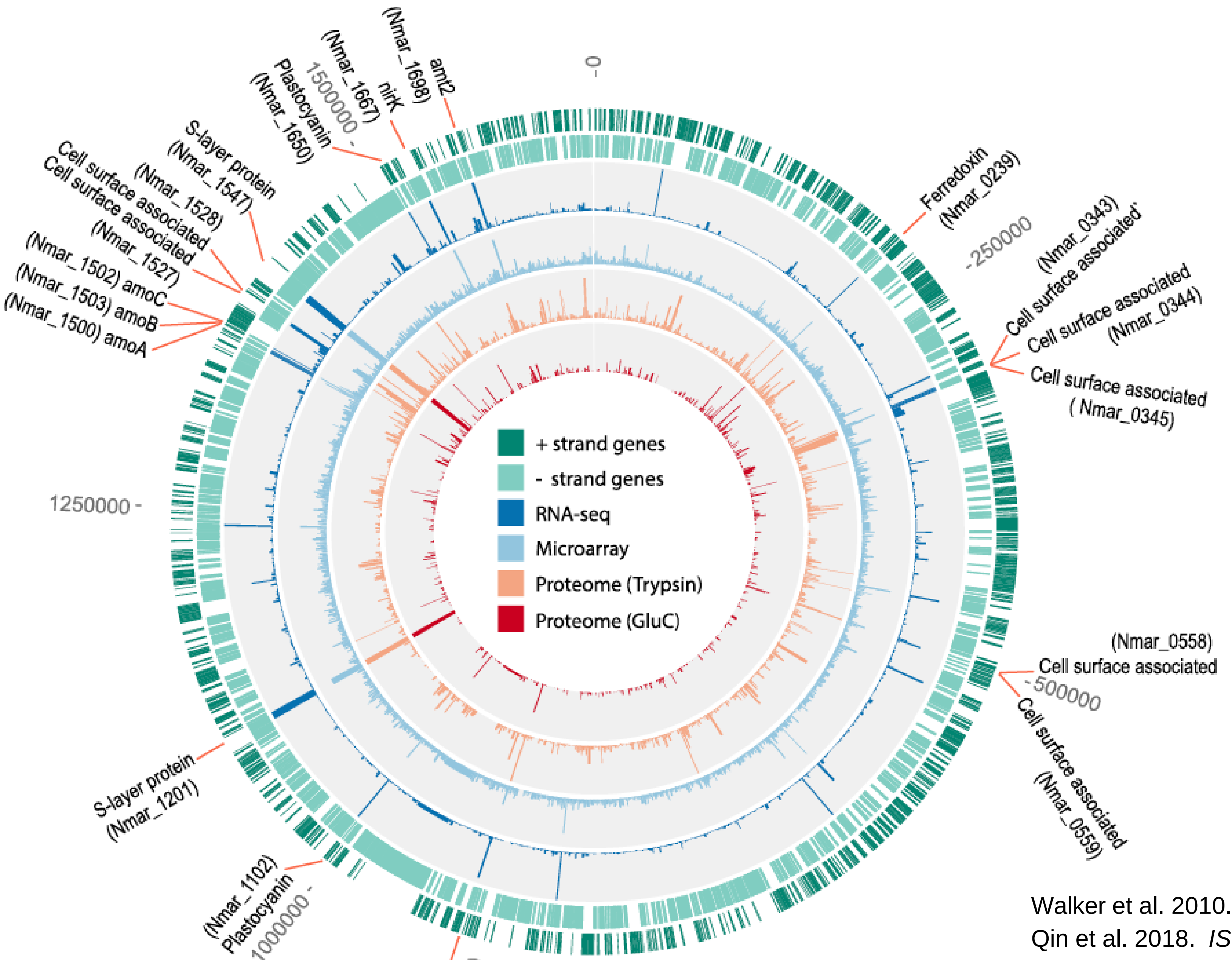
Brochier-Armanet et al. 2008. Nature Rev. Microbiol.; Parks et al. 2017. Nature Microbiol.

Archaeal ammonia oxidizers dominant in most environments

AOA /AOB >100 in most terrestrial and marine systems

Current understanding of supporting biochemistry

Framework provided by the *N. maritimus* genome (1.62 Mbp) & physiology



Walker et al. 2010. *PNAS*
Qin et al. 2018. *ISME J*

Biochemistry – as inferred from genome & proteome

Pathway for ammonia oxidation

- A distant variant of ammonia monooxygenase the <u>only</u> homolog to the well characterized bacterial system
- Many copper proteins, including variants of nitrite reductase

$$NO_2^- + 1\ e^- \longrightarrow NO$$

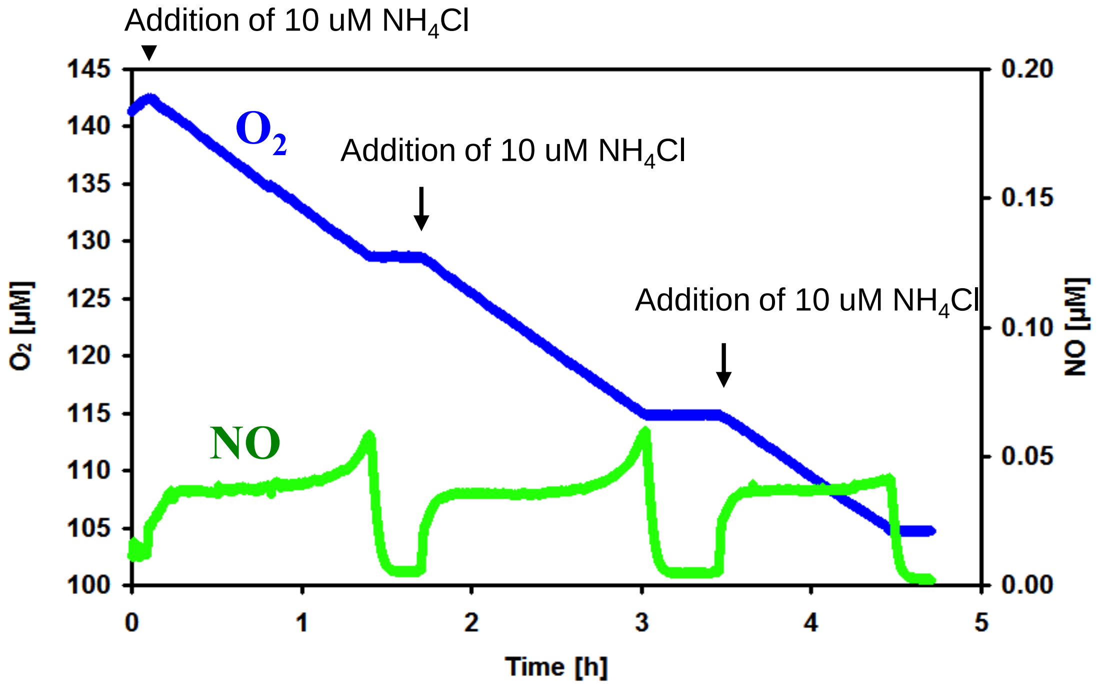
Pathway CO_2 fixation

- Evidence for variant of hydroxypropionate/hydroxybutyrate pathway

An essential role for nitric oxide in archaeal ammonia oxidation

Nitric oxide – an intermediate or reactant in AOA ammonia oxidation

- Scavengers of NO (PTIO) immediately suppress ammonia oxidation

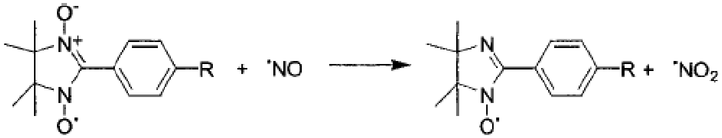


$$NH_3 + O_2 + 2e^- + 2H^+ \xrightarrow[\text{Ammonia monooxygenase}]{} H_2O + NH_2OH \xrightarrow[?]{} NO\ ? \xrightarrow[?]{} NO_2^-$$

Martens-Habbena et al. 2015. Environ. Microbiol.

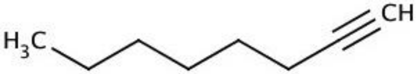
Served for development of thaumarchaeal and bacterial-specific inhibitors of ammonia oxidation

Selective inhibition of AOA



PTIO (2-phenyl-4,4,5,5, tetramethylimidazoline-1-oxyl 3-oxide), an NO scavenger

Selective inhibition AOB

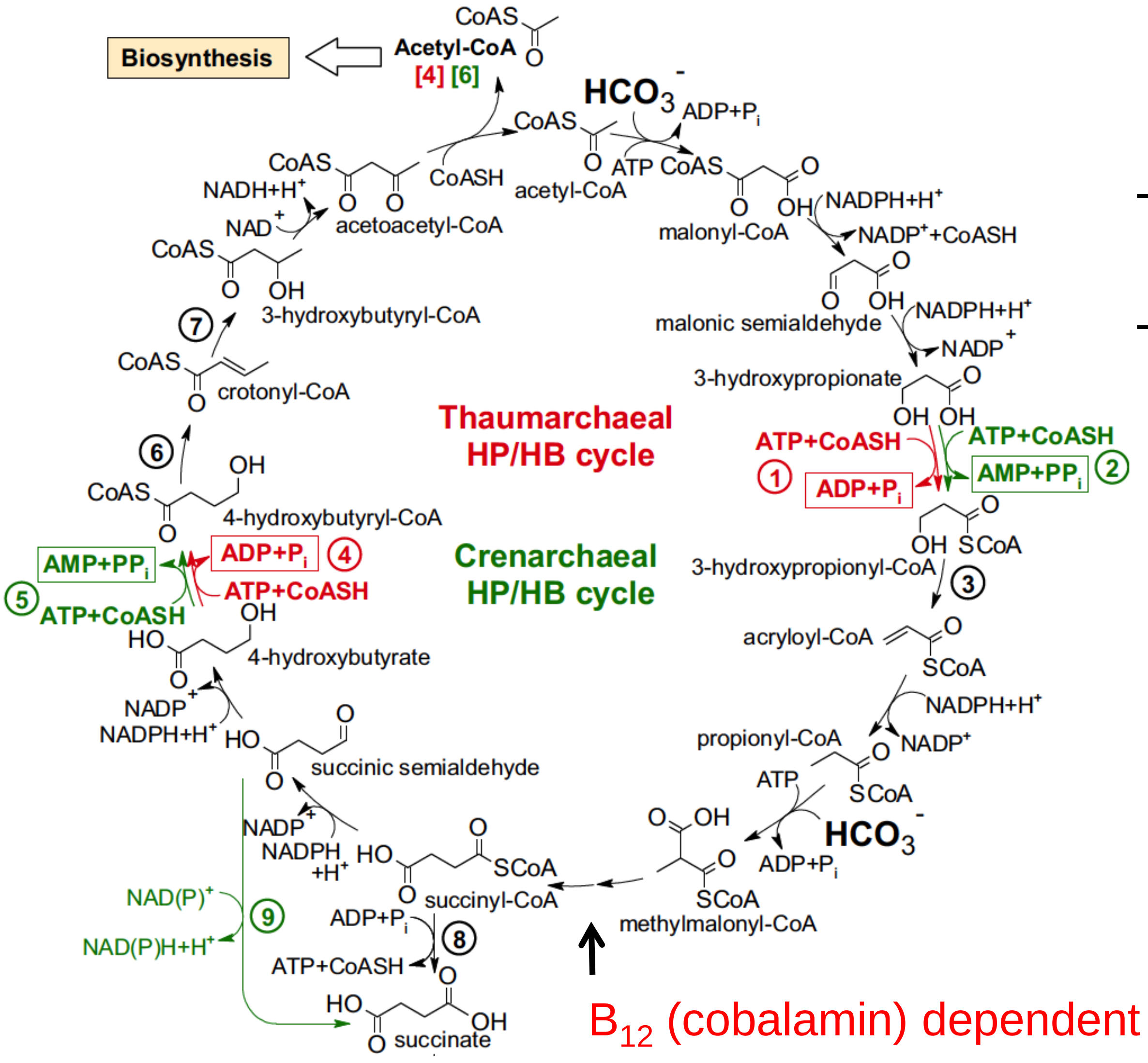


Octyn, mechanism-based irreversible inactivation of bacterial ammonia monooxygenase

Martens-Habbena et al. 2015. Environ. Microbiol.; Taylor et al. 2015. Appl. Environ. Microbiol.

CO_2 fixation via a highly efficient modification of HP/HB cycle

most efficient among characterized aerobic pathways

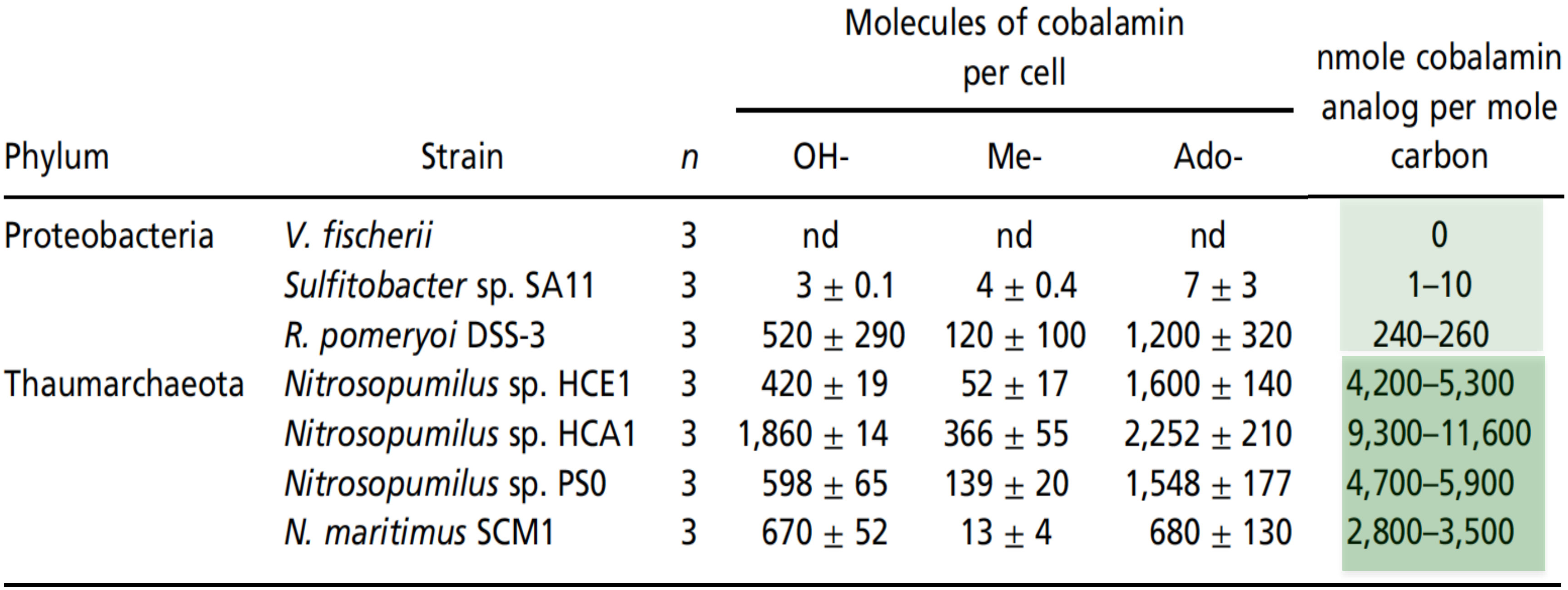


B_{12} (cobalamin) dependent mutase

Pathway Variant	~P per g biomass
AOA HP/HP	0.09
Calvin-Benson	0.12

M. Könneke et.al. 2014. *PNAS* 111: 8239-8244

Extremely high cellular quotas of cobalamin in *Nitrosopumilus* strains



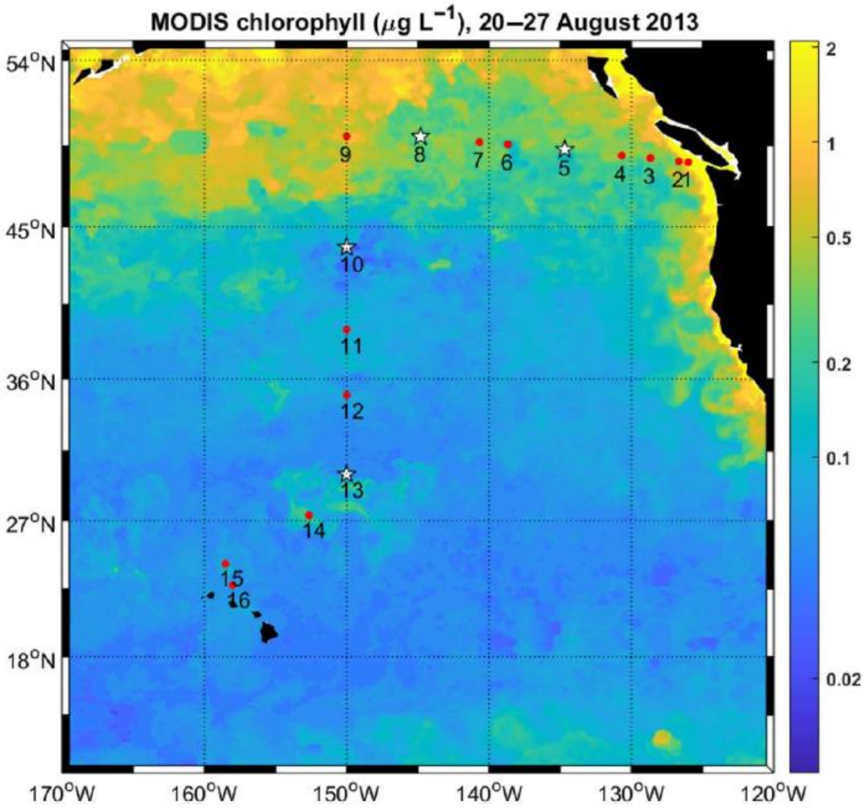
Phylum	Strain	*n*	Molecules of cobalamin per cell			nmole cobalamin analog per mole carbon
			OH-	Me-	Ado-	
Proteobacteria	*V. fischerii*	3	nd	nd	nd	0
	Sulfitobacter sp. SA11	3	3 ± 0.1	4 ± 0.4	7 ± 3	1–10
	R. pomeryoi DSS-3	3	520 ± 290	120 ± 100	1,200 ± 320	240–260
Thaumarchaeota	*Nitrosopumilus* sp. HCE1	3	420 ± 19	52 ± 17	1,600 ± 140	4,200–5,300
	Nitrosopumilus sp. HCA1	3	1,860 ± 14	366 ± 55	2,252 ± 210	9,300–11,600
	Nitrosopumilus sp. PS0	3	598 ± 65	139 ± 20	1,548 ± 177	4,700–5,900
	N. maritimus SCM1	3	670 ± 52	13 ± 4	680 ± 130	2,800–3,500

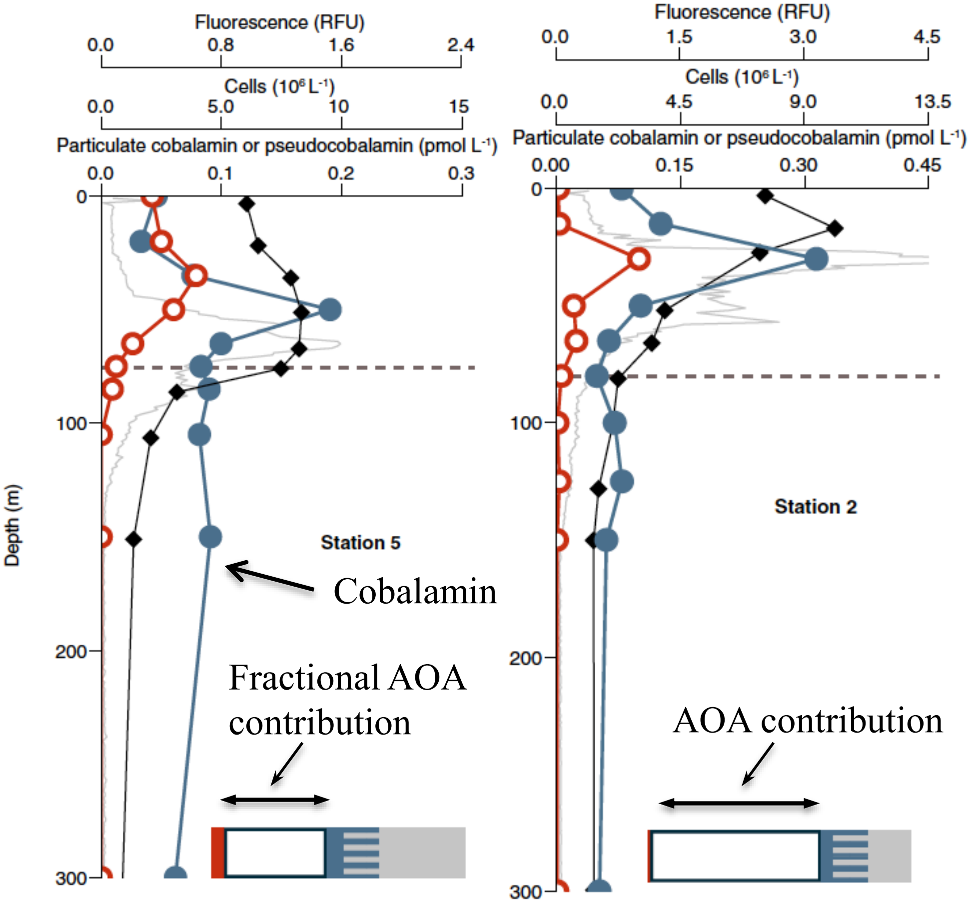
Heal et al. 2017. *PNAS* 114:364

AOA can contribute to more than half the B_{12} pool in North Pacific

In the surface ocean most eukaryotic algae are cobalamin auxotrophs

2013 Cruise track of the RV Kilo Moana





Cobalamin

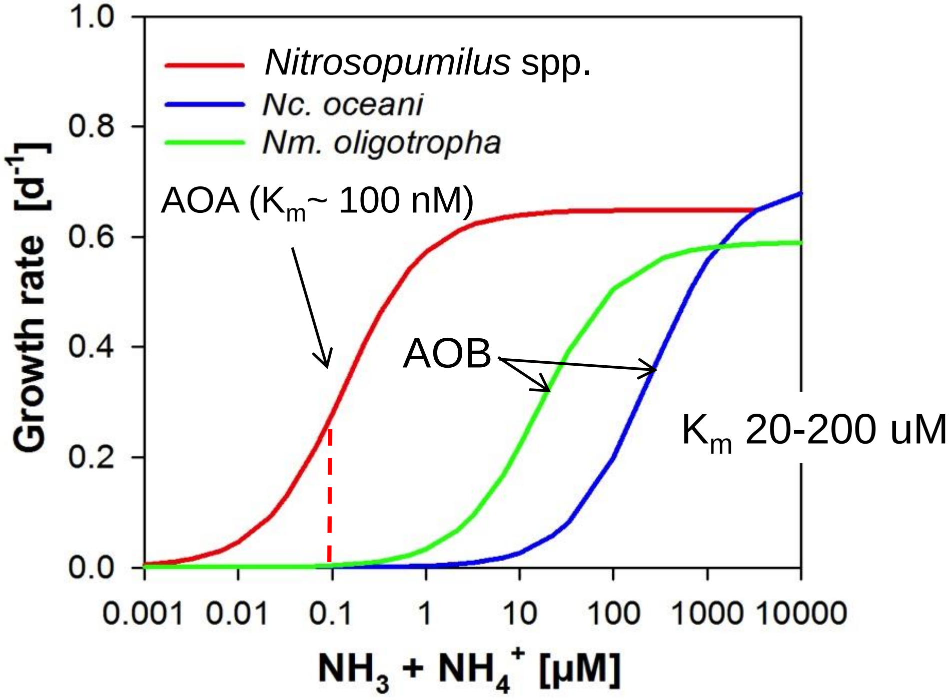
Fractional AOA contribution

AOA contribution

Heal et al. 2017. PNAS

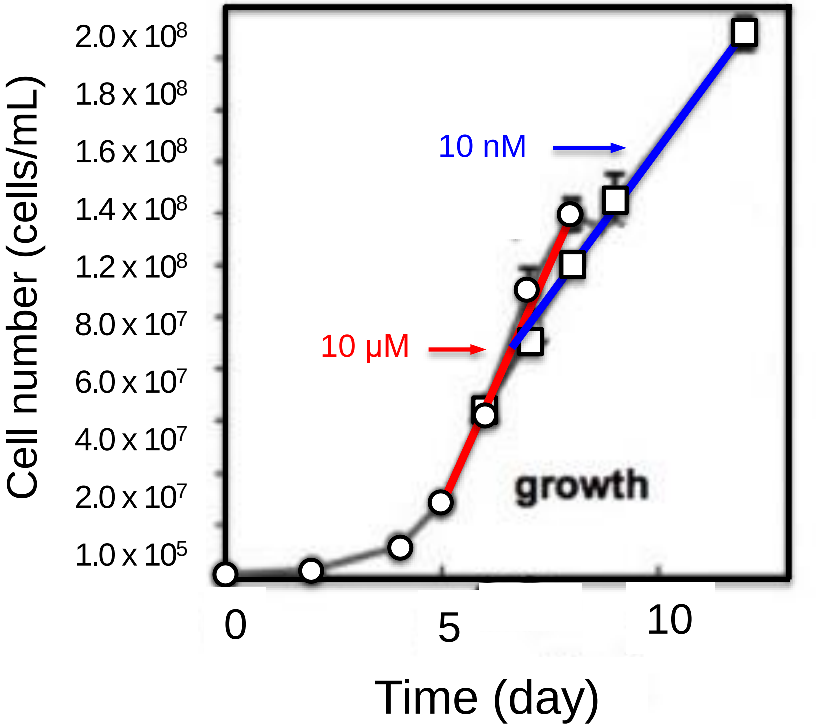
Physiological basis for the remarkable environmental success of AOA

exceptionally high affinities for ammonia and oxygen



Martens-Habbena et al. 2009 *Nature*; Qin et al. 2017 Env. Microbiol. Rep.

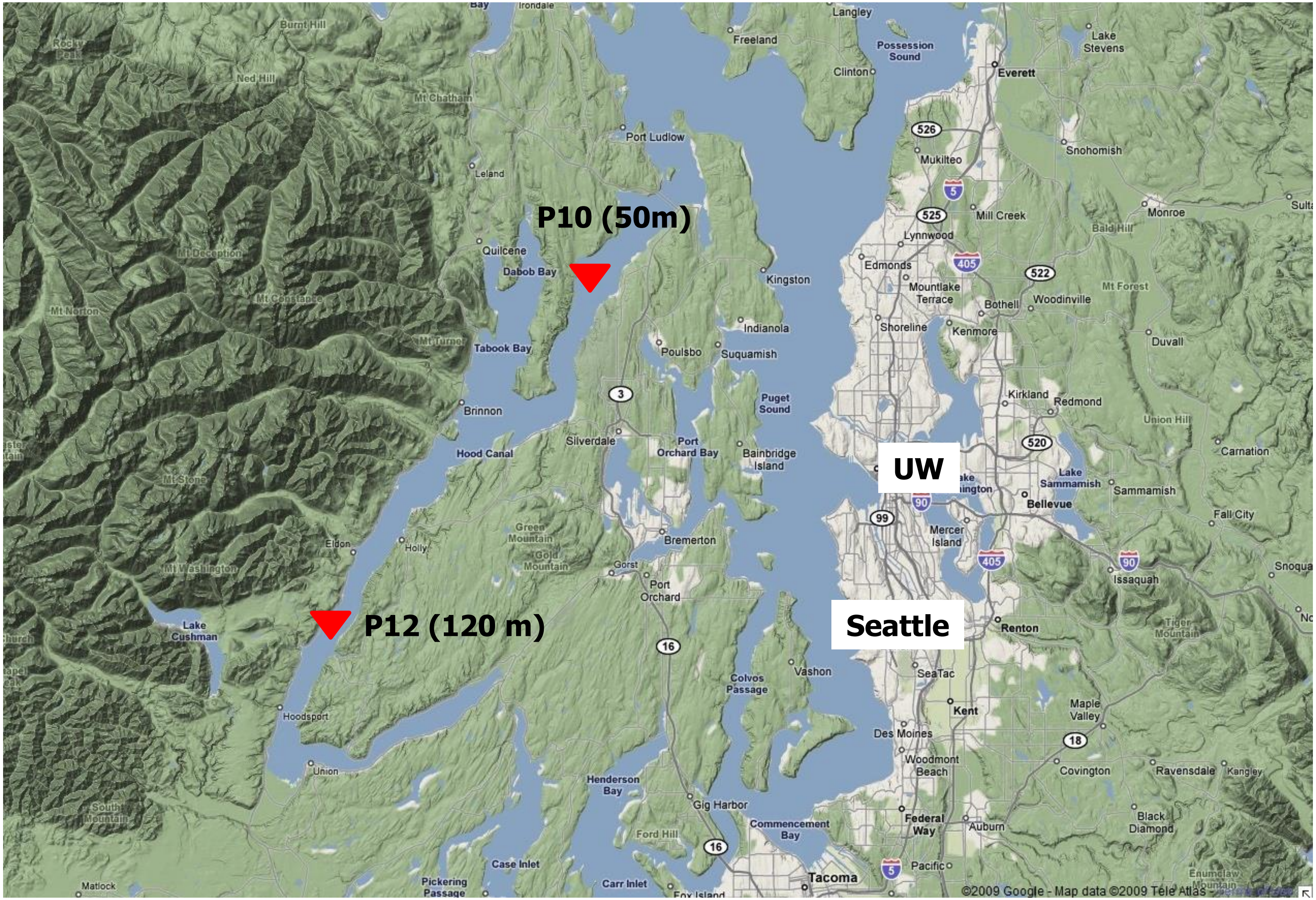
Active growth at low nM concentrations of ammonia



Nakagawa & Stahl. 2013. Appl. Environ. Microbiol.

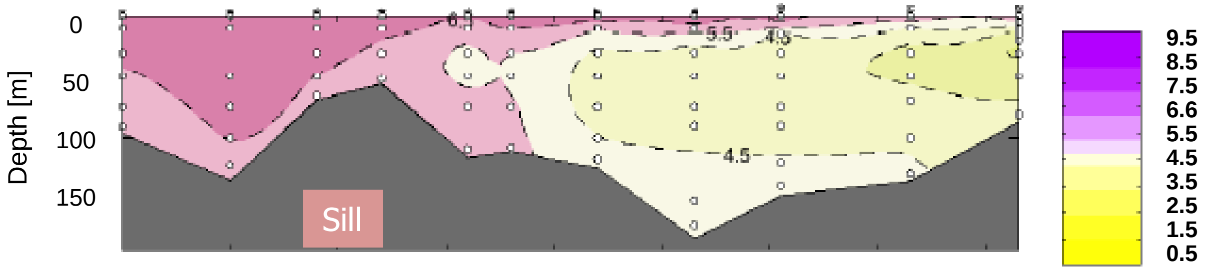
Coastal marine distribution, abundance, and activity

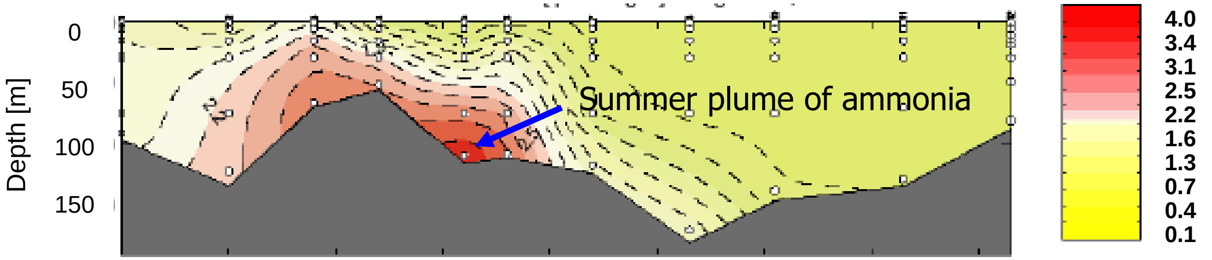
Hood Canal field sites



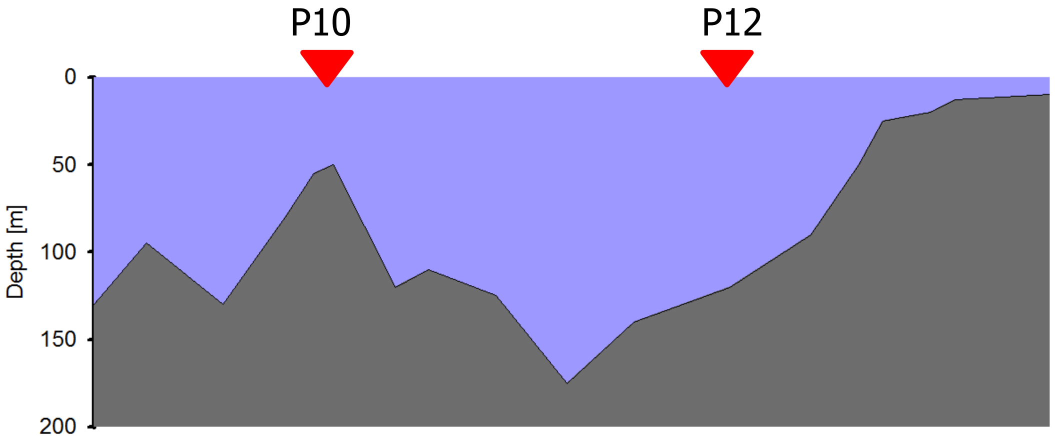
Sampling sites and water chemistry in Hood Canal

Spatial structure of O_2 and NH_4^+ (summer)

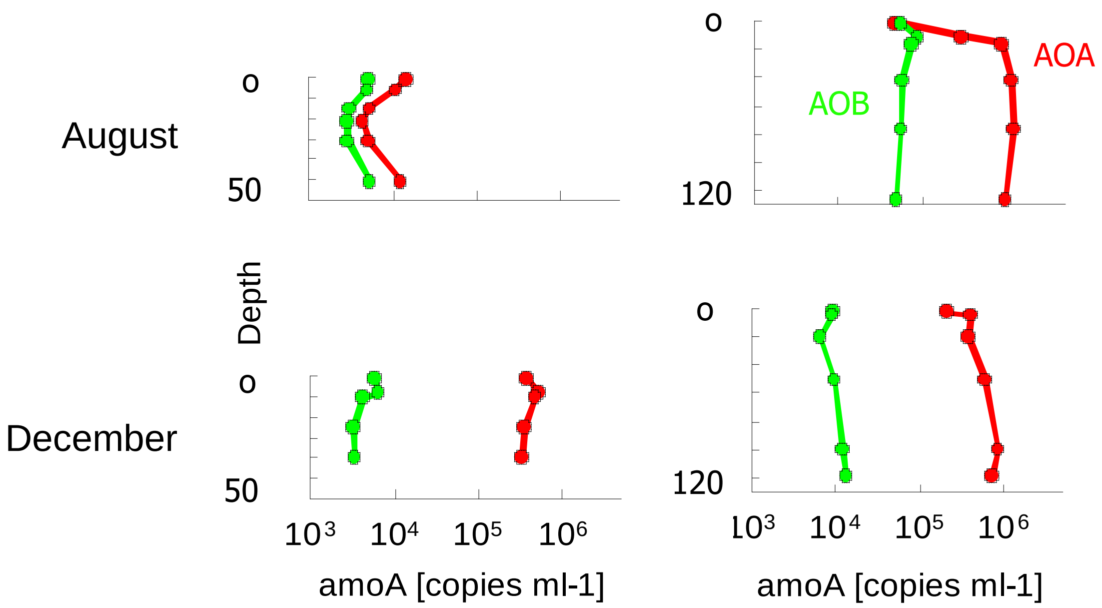

Oxygen [mg/L] August 21, 1996


Ammonium [ug/L] August 21, 1996

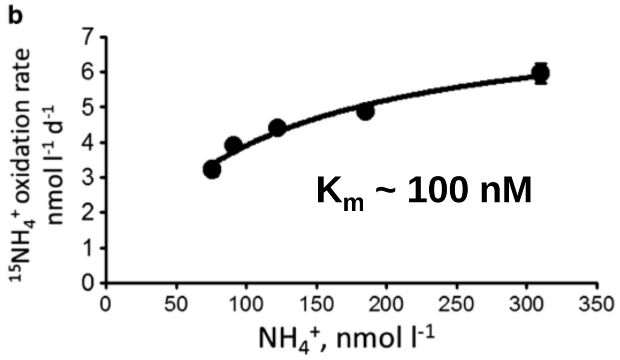
Summer plume of ammonia

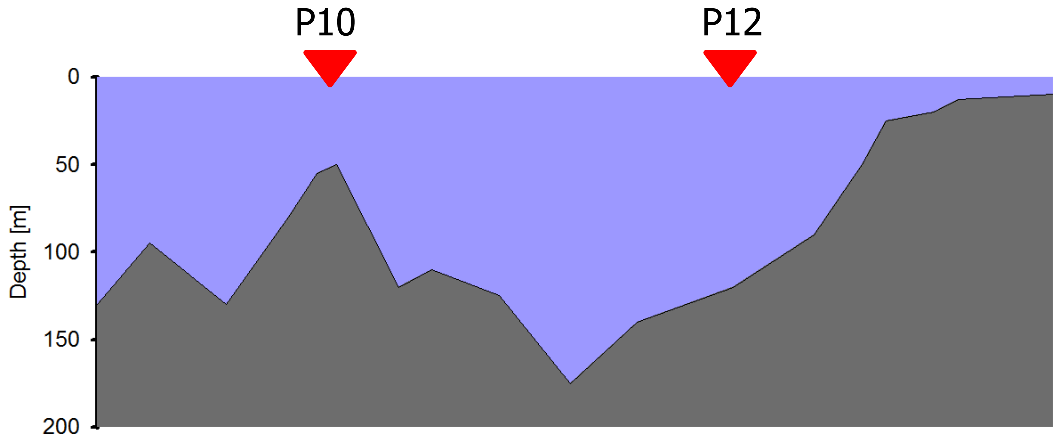


AOA dominate in numbers and process in these coastal waters



Coastal waters *in situ* ammonia oxidation kinetics





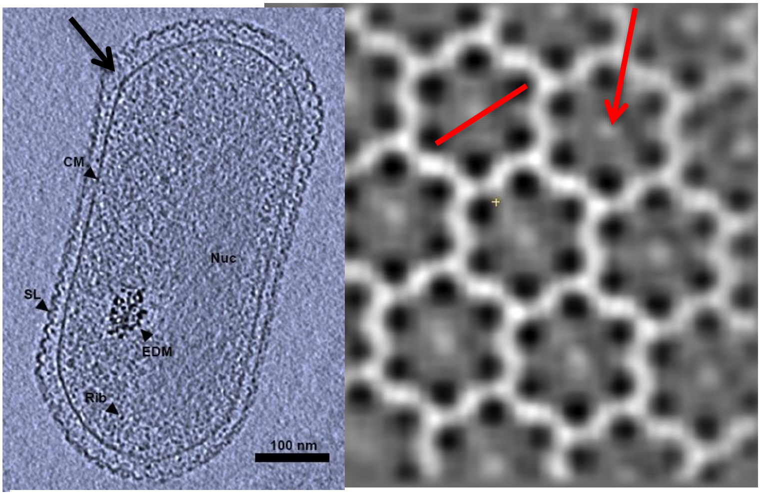
Central North Pacific

K_m ~ 10 nM

Horak et al. *ISME J.* 2013, Urakawa et al. *Limnol. Oceanogr*. 2014

Novel ammonia oxidation system integrated with protein surface layer (S-layer)

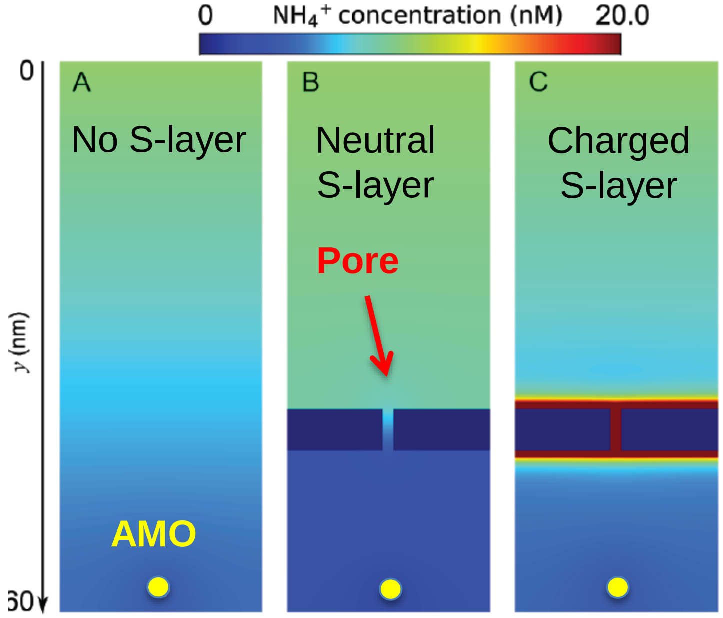
Modeling shows that charged S-layer facilitates ammonia acquisition & oxidation

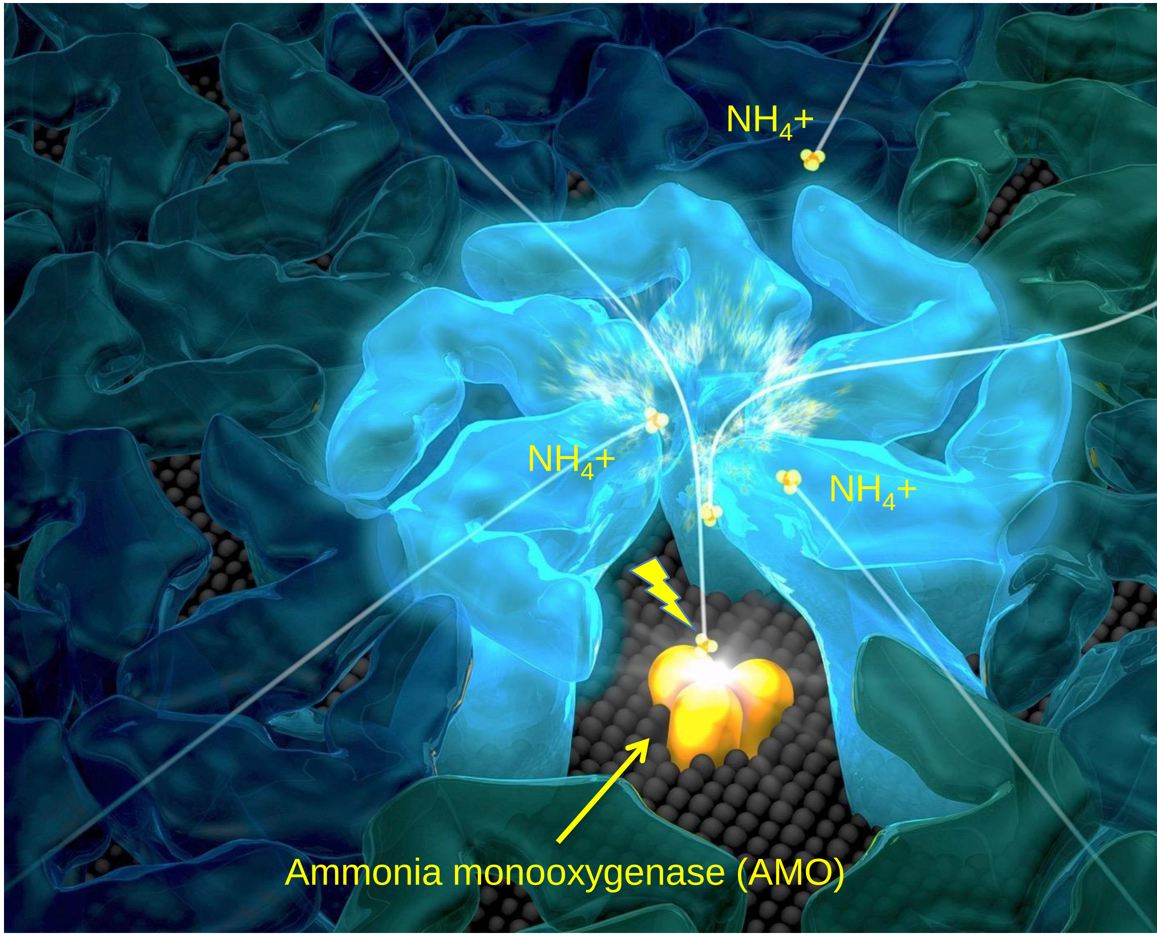


S-layer

16.34 nm unit diameter

1.5 nm central Pore

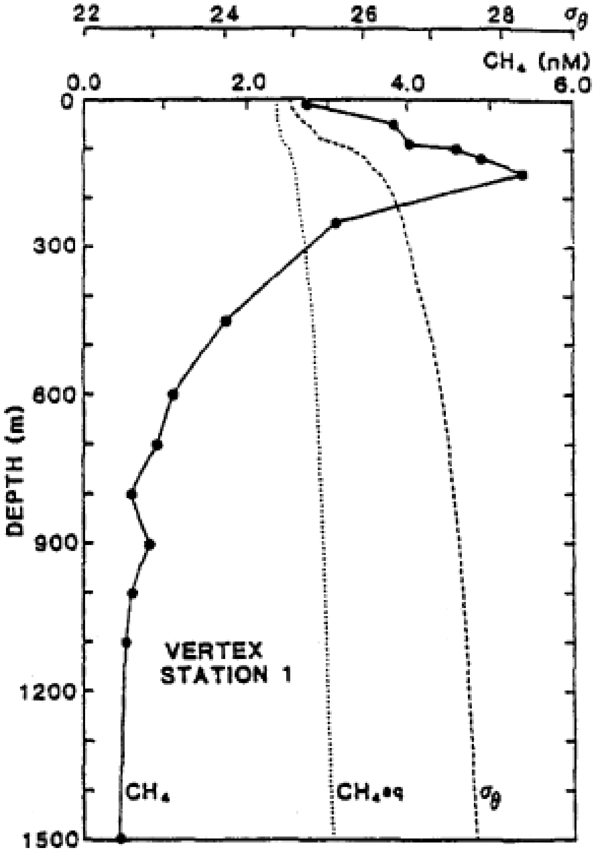




Po-Nan Li, Jonathan Herrmann, Frederic Poitevin, Rasika Ramdasi, Bradley B. Tolar, John R. Bargar, David A. Stahl, **Grant J. Jensen**, Christopher A. Francis, **Soichi Wakatsuki**, and **Henry van den Bedem**. ISME Journal in press

Ocean Methane Paradox

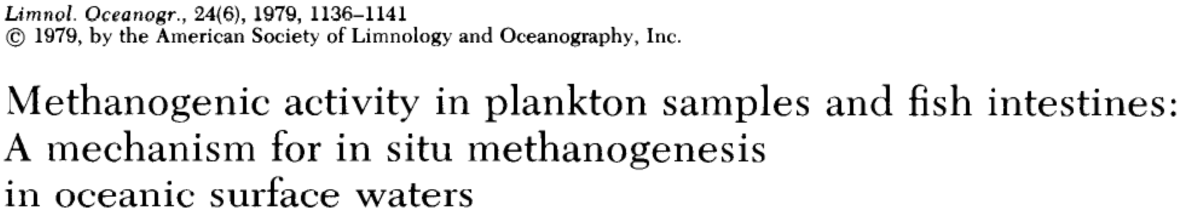
The surface ocean is supersaturated in CH_4 with respect to the atmosphere. Methanogenesis occurs only under strict anoxic conditions, so its occurrence and apparent production in oxic waters to an extent that produces methane supersaturation is termed the “Ocean Methane Paradox”. W. S. Reeburgh. 2007. Oceanic Methane Biogeochemistry. Chem. Rev.107: 486-513.



Tilbrook, B.D. and D.M. Karl. 1995. Methane sources, distributions and sinks from California coastal waters to the oligotrophic North Pacific gyre. Marine Chemistry 49: 5 l-64.

Methanogen microniches?

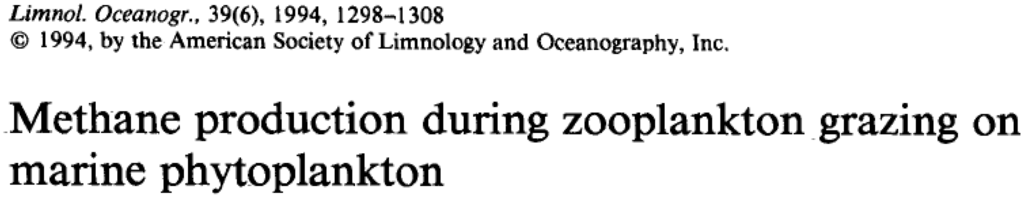
Oremland, 1979



Limnol. Oceanogr., 24(6), 1979, 1136–1141
© 1979, by the American Society of Limnology and Oceanography, Inc.

Methanogenic activity in plankton samples and fish intestines:
A mechanism for in situ methanogenesis
in oceanic surface waters

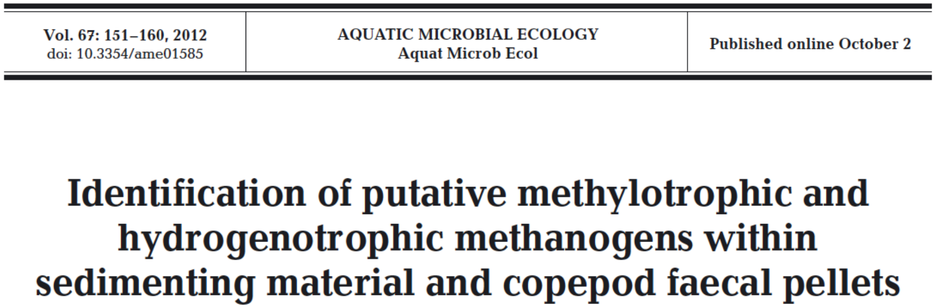
de Angelis and Lee, 1994



Limnol. Oceanogr., 39(6), 1994, 1298–1308
© 1994, by the American Society of Limnology and Oceanography, Inc.

Methane production during zooplankton grazing on marine phytoplankton

Ditchfield et al. 2012

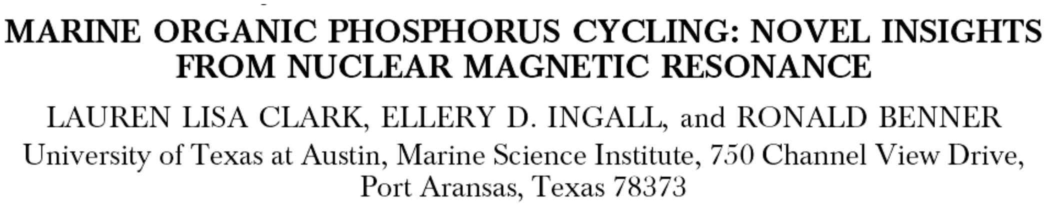


Vol. 67: 151–160, 2012 doi: 10.3354/ame01585	AQUATIC MICROBIAL ECOLOGY Aquat Microb Ecol	Published online October 2

Identification of putative methylotrophic and hydrogenotrophic methanogens within sedimenting material and copepod faecal pellets

NMR characterization of dissolved organic phosphorous in Central Pacific

DOP is a significant fraction of total P

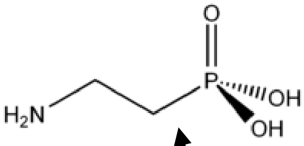


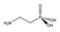
MARINE ORGANIC PHOSPHORUS CYCLING: NOVEL INSIGHTS FROM NUCLEAR MAGNETIC RESONANCE

LAUREN LISA CLARK, ELLERY D. INGALL, and RONALD BENNER
University of Texas at Austin, Marine Science Institute, 750 Channel View Drive,
Port Aransas, Texas 78373

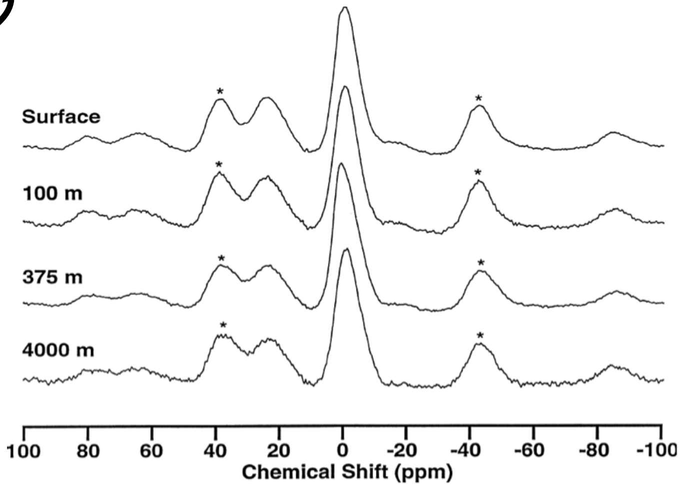
Phosphonates (~25%)

Phosphate esters (~75%)





Organic phosphates containing a C-P bond

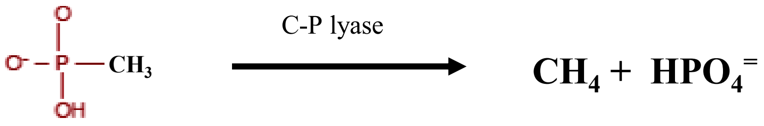


The phosphonate-methane hypothesis

Karl et al. *Nature Geoscience.* July 2008

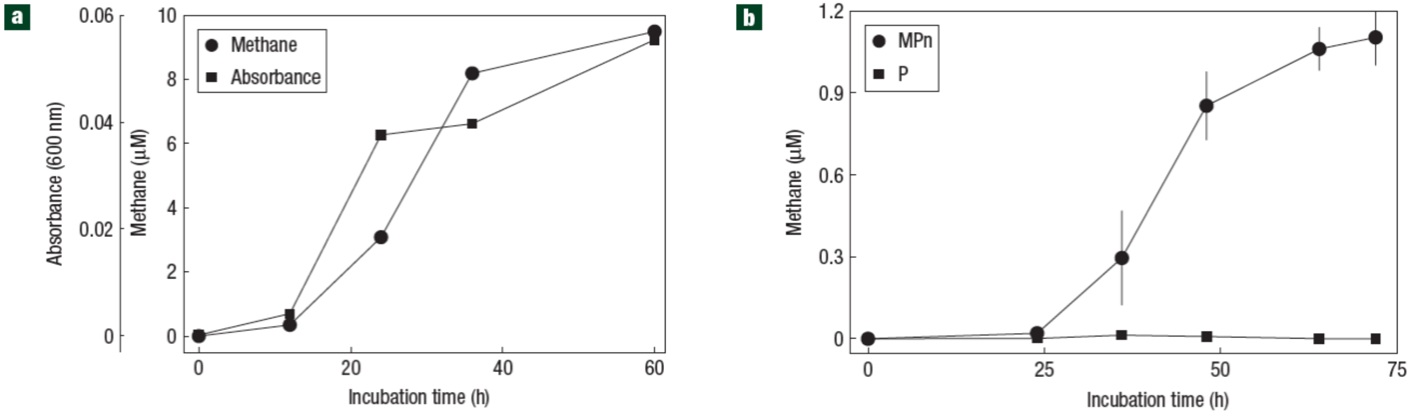
“We hypothesize that methylphosphonate (Mpn) cycling (its coupled production and decomposition) is a pathway for the aerobic formation of methane in marine ecosystems. The […] ‘missing’ source term needed to reconcile the observed methane supersaturations that sustain a net flux of methane from the ocean to the atmosphere.”

Caveat - However, MPn only available from chemical synthesis**.**



$$\mathrm{O^{-}-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{P}}-CH_3} \xrightarrow{\text{C-P lyase}} \mathrm{CH_4 + HPO_4^{=}}$$

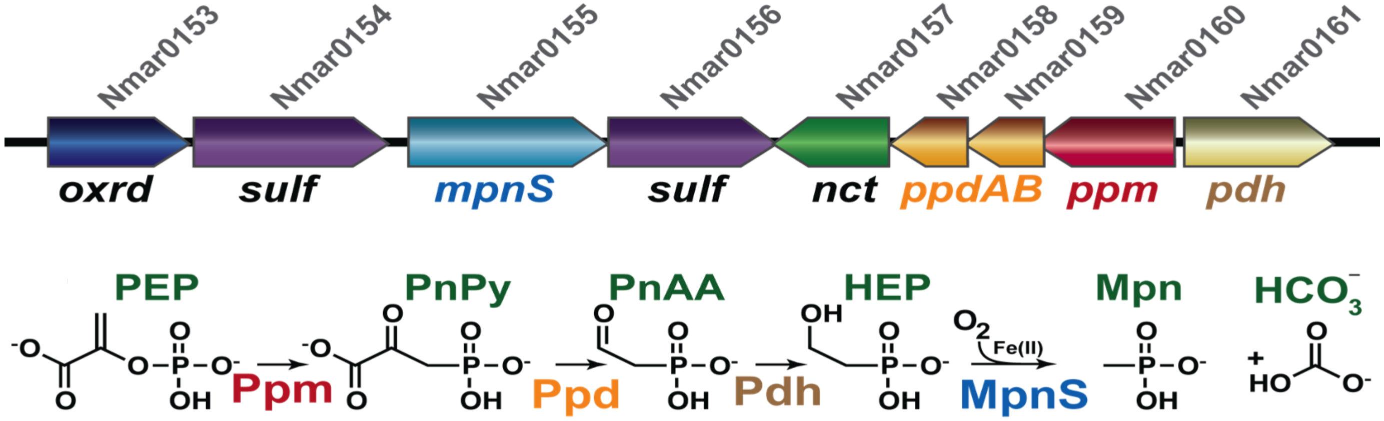
Methylphosphonate



Mpn added to seawater is used as a phosphorous source for growth of marine plankton, and its assimilation correlated with methane production.

Pathway for methylphosphonate synthesis in *N. maritimus*

Proximity to genes encoding EPS synthesis (e.g., sugar nucleotidyl transferase & glycosyl transferase) suggests role in surface modification



PEP, phosphoenolpyruvate
PnPy, phosphonopyruvate
PnAA, phosphonoacetaldehyde
HEP, hydroxyethylphosphonate
MPn, methylphosphonate
HMP, hydroxymethylphosphonate
HPP, hydroxypropylphosphonate
HCO_3^-, bicarbonate; HCO_2^-, formate.

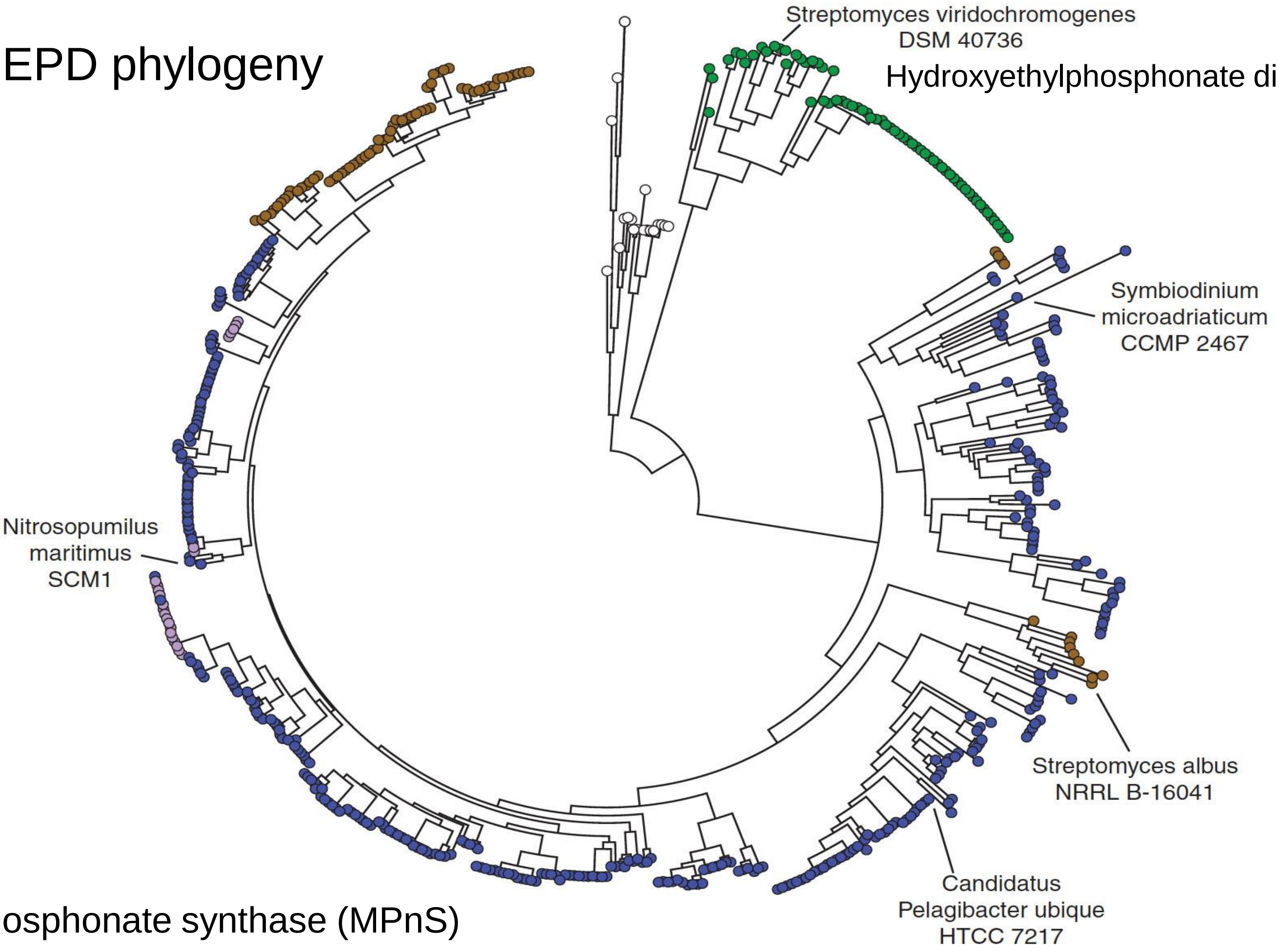
phosphonopyruvate mutase (*ppm*)
phosphonopyruvate decarboxylase (*ppdAB*)
phosphonoacetaldehyde dehydrogenase (*pdh*)
methylphosphonate synthase (*mpnS*)

Metcalf et al. 2012. *Science* 337:1104

MPnS widely distributed among abundant marine microorganisms

but, so far only found in marine environments

MPnS & HEPD phylogeny



Hydroxyethylphosphonate dioxygenase (HEPD)

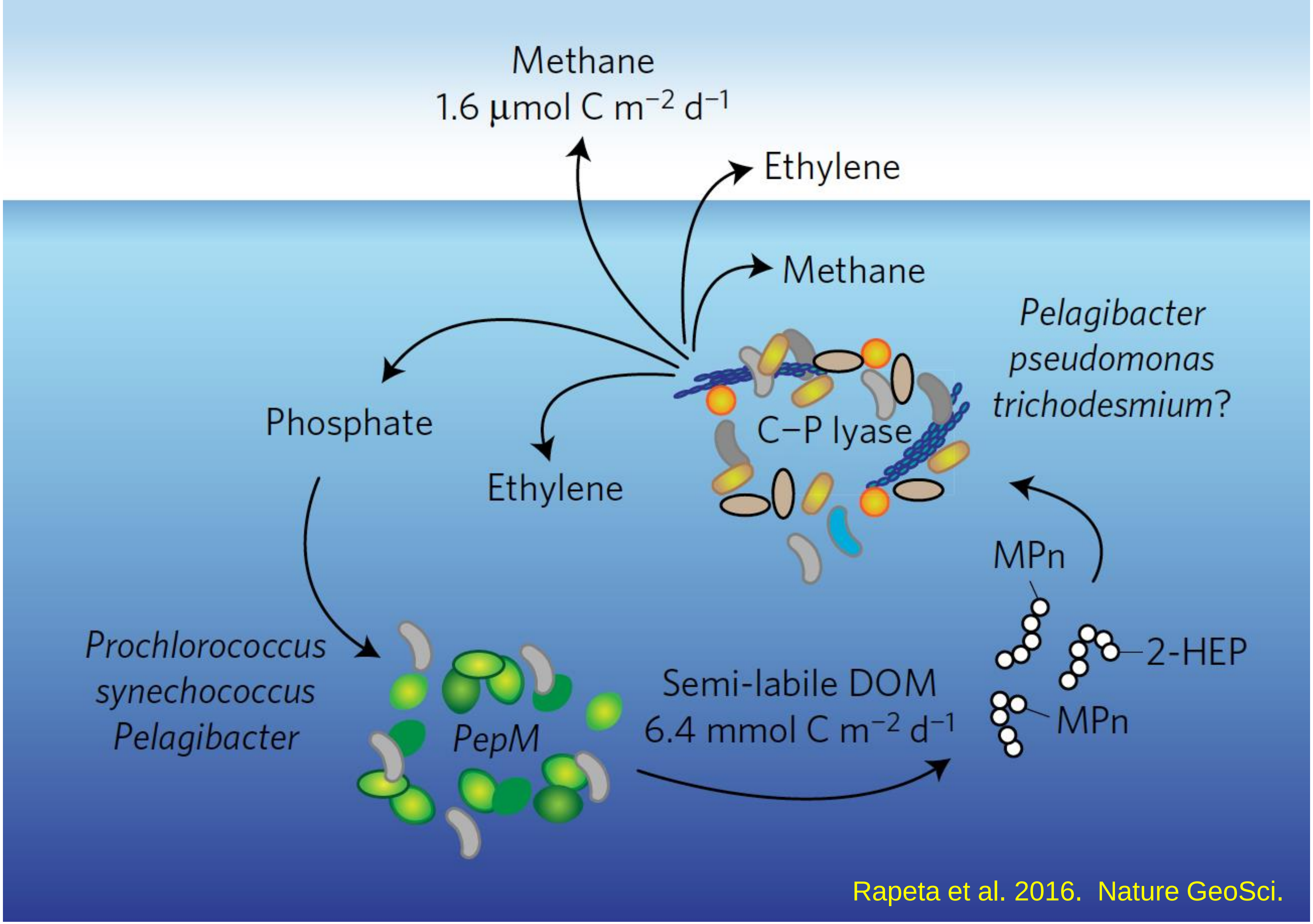
● ●

Methylphosphonate synthase (MPnS)

● ●

Born et al. 2017. *Science* 358: 1336

Phosphonates comprise about 20% of P in semi-labile carbohydrate pool



Rapeta et al. 2016. Nature GeoSci.

A few concluding summary points

Today there is an even a greater role for organismal science in environmental microbiology

As today’s example - identification, isolation and physiological characterization of AOA

- Established their biogeochemical significance, archaea now recognized to mediate the rate-controlling step of nitrification in most environments
- Identified a novel phosphonate biochemistry as a plausible source of oceanic surface methane flux
- Identified a major source of vitamin B_{12} in the marine environment, essential for most algae (linking nitrogen and carbon cycles)
- Served for development of selective inhibitors, of basic and applied utility

However, because of that lifestyle, this important population was long overlooked using standard cultivation methods (which generally selects for “weedy” species)

Many thanks to all the students, postdocs, & technicians

John Urbance
Berdena Flesher
Chuzhao Lin
Matt Kane
Richard Devereux
Julie Stromley
Lutgarde Raskin
Barth Smets
Rudi Amann
Michi Wagner
Barbara MacGregor
Richard Sharp
Norman Fry
Sue Fishbain
Brad Jackson
Sam Webb
Meredith Hullar
Slil Siripong
Sergey Stolyar
Paul Berube
Nic Pinel
Wei Qin
Jason Flowers
Kelley Meinhardt
Nick Elliot

Birte Meyer
Jennifer Becker
Liz Alm
Lee Krumholz
Joel Dore
Rebecca Key
Lars Poulsen
Agnes Odenyo
Dan Noguera
Jay Regan
Bill Capman
Simon Toze
Bruce Mobarry
Brett Baker
Duane Moser
Greg Brusseau
Y. Koizumi
Andy Schramm
Heidi Gough
Anne Bernhard
Jesse Dillon
Michelle Starke
Kristina Hillesland
Willm Martens-Habbena
Glenn Dulla
Lara Rajeev

Cheri Ziemer
Jodi Flax
Gina Berardesco
Kerstin Sahm
Dror Minz
Stefan Green
John Kelly
Laura Sappelsa
Ben van Mooy
Wen-Tso Liu
Andreas Teske
Hidetoshi Urakawa
Peter Noble
Said El Fantroussi
Takko Nakagawa
Seana Davidson
Jeremy Dodsworth
Uffe Thomsen
Laura Smoot
Jim Smoot
Martin Koenneke
Chris Walker
Jose de La Torre
Peter Andeer
Tony Bertagnolli

Funding - NSF DOE JGI