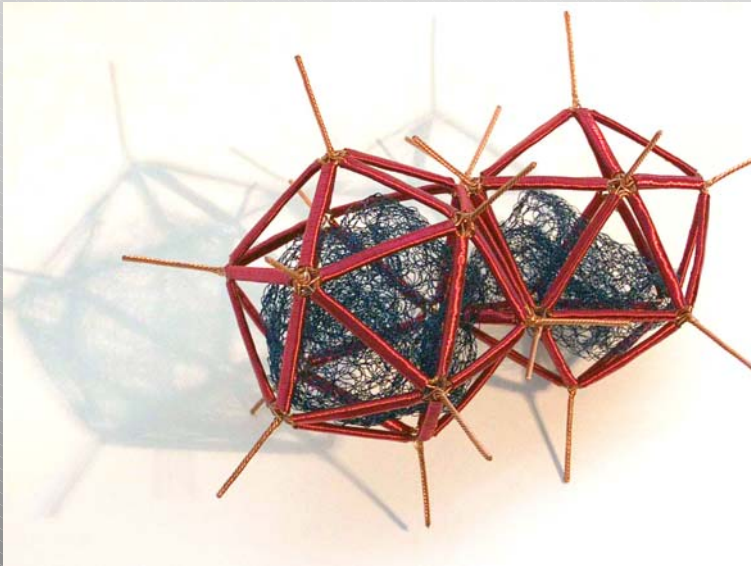


Viral biodiversity, ecology, evolution, & a little coral stuff...



Phage art by Jennifer Jo Walrath

Forest Rohwer

**San Diego State University
&
Center for Microbial Sciences**



Ecological roles of phage

- **Most abundant predators**
 - ~ 10^7 viruses per ml surface seawater
 - estimated 10^{31} viruses on the planet
- **Major players in global C cycling**
- **Increase microbial diversity**
 - "Kill-the-Winner"
- **Transduction and lysogenic conversion**
 - 10^{25} - 10^{28} base pairs of DNA per year in the oceans
 - cholera toxin

Cloning viral communities

Filter to remove microbes (0.16 μm)



Concentrate using a 100 kD TFF



Purify phage using DNase, RNase, & CsCl



Extract phage DNA



Linker-Amplified Shotgun Libraries (LASLs)

David Mead -



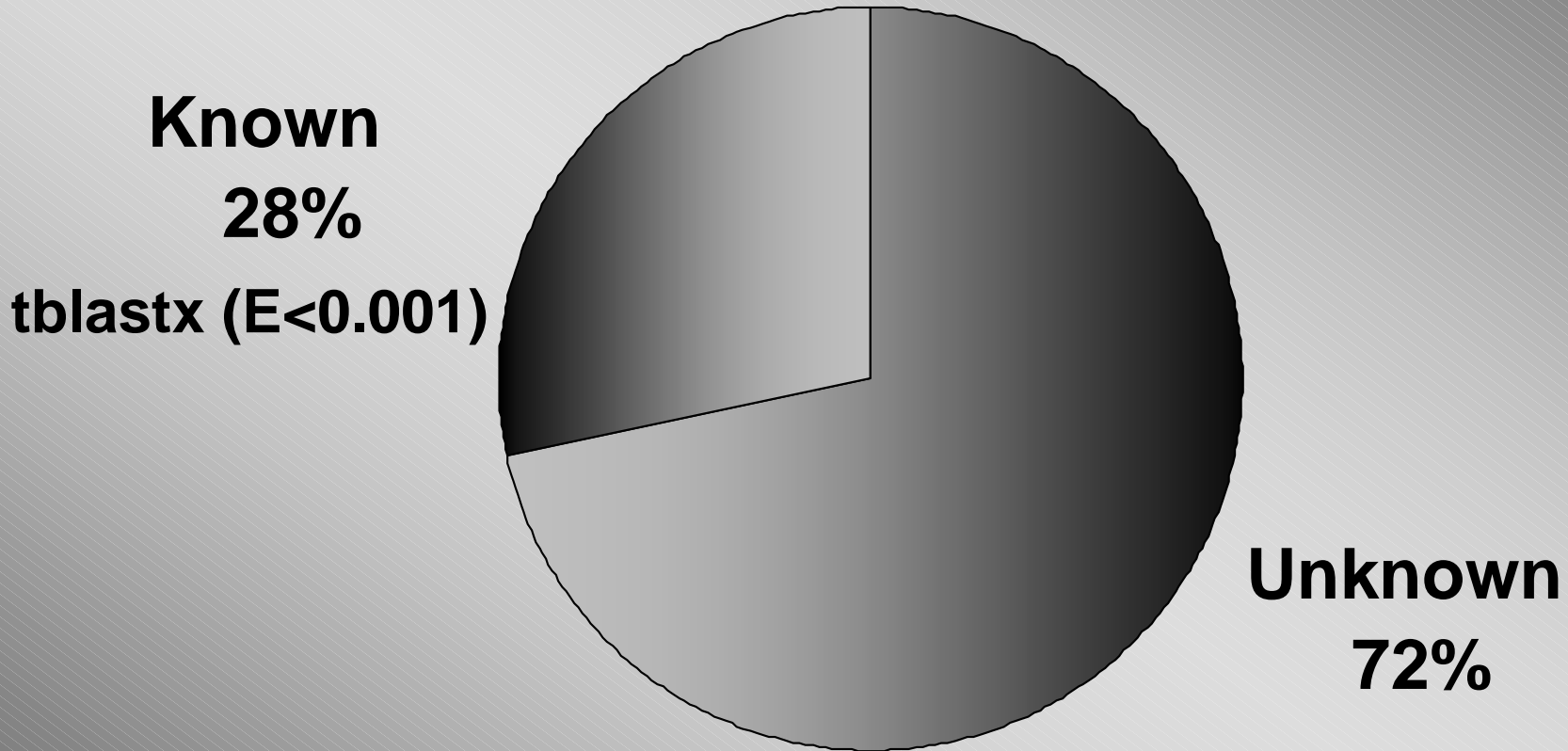
Sequence

Breitbart et al., (2002) "Marine water viruses" PNAS. 99:14250-14255.

Breitbart et al., (2003) "Fecal viruses" J Bact. 85 (20). 6220-6223.

Breitbart et al., (2004) "Marine sediment viruses" Proc Royal Society B. 271. 565-574.

Most uncultured phage sequences are "Unknowns"

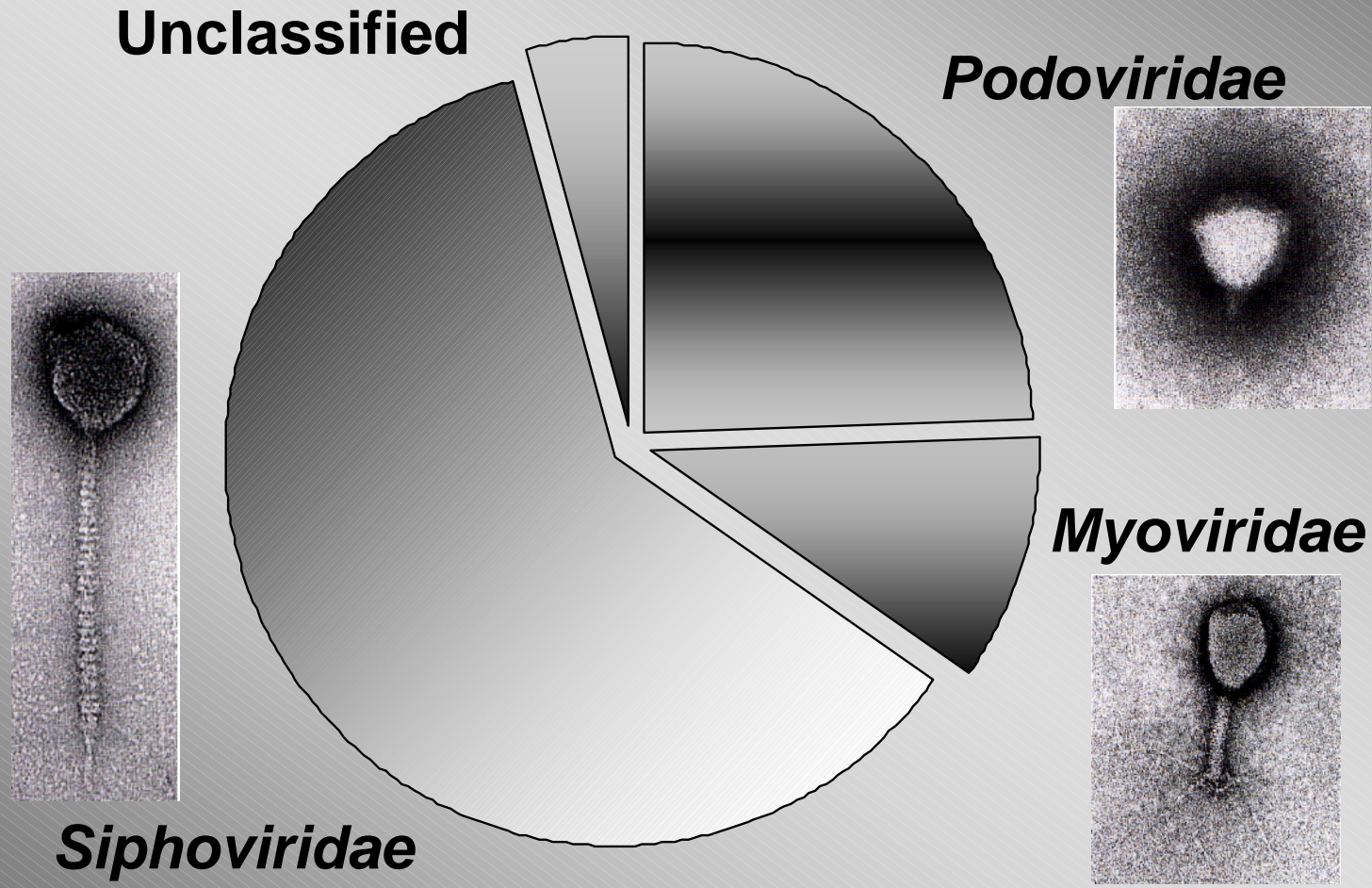


<0.0002% of the global phage metagenome has been sampled

Rohwer (2003) Global phage diversity. Cell. 113.

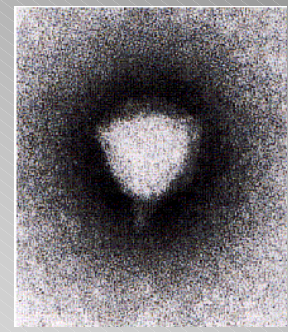
> preview for Pedulla et al. (2003) Origins of highly mosaic mycobacteriophage genomes

Siphophage are the most common type of phage



Unclassified

Podoviridae

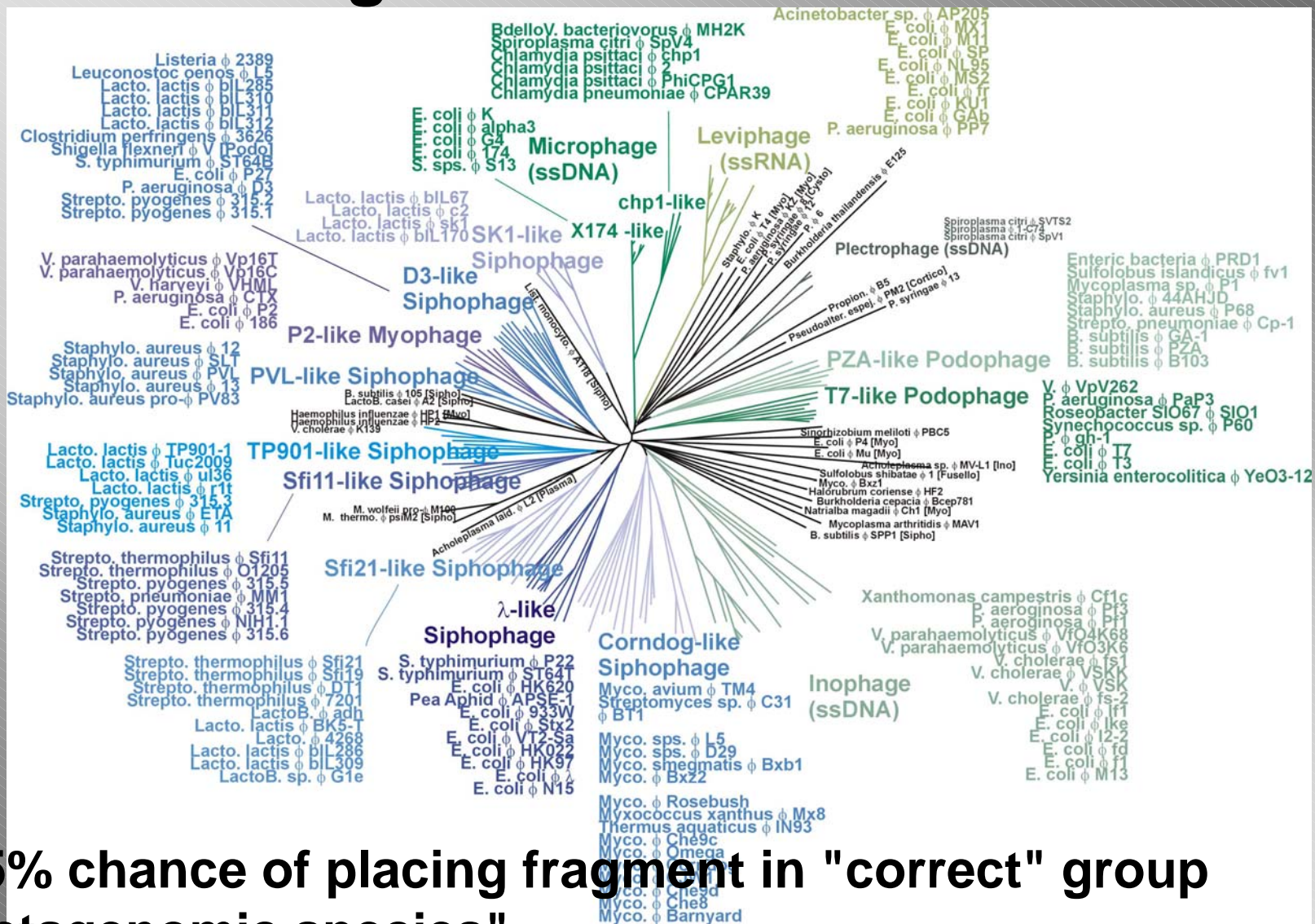


Siphoviridae

Myoviridae



Phage Proteomic Tree II

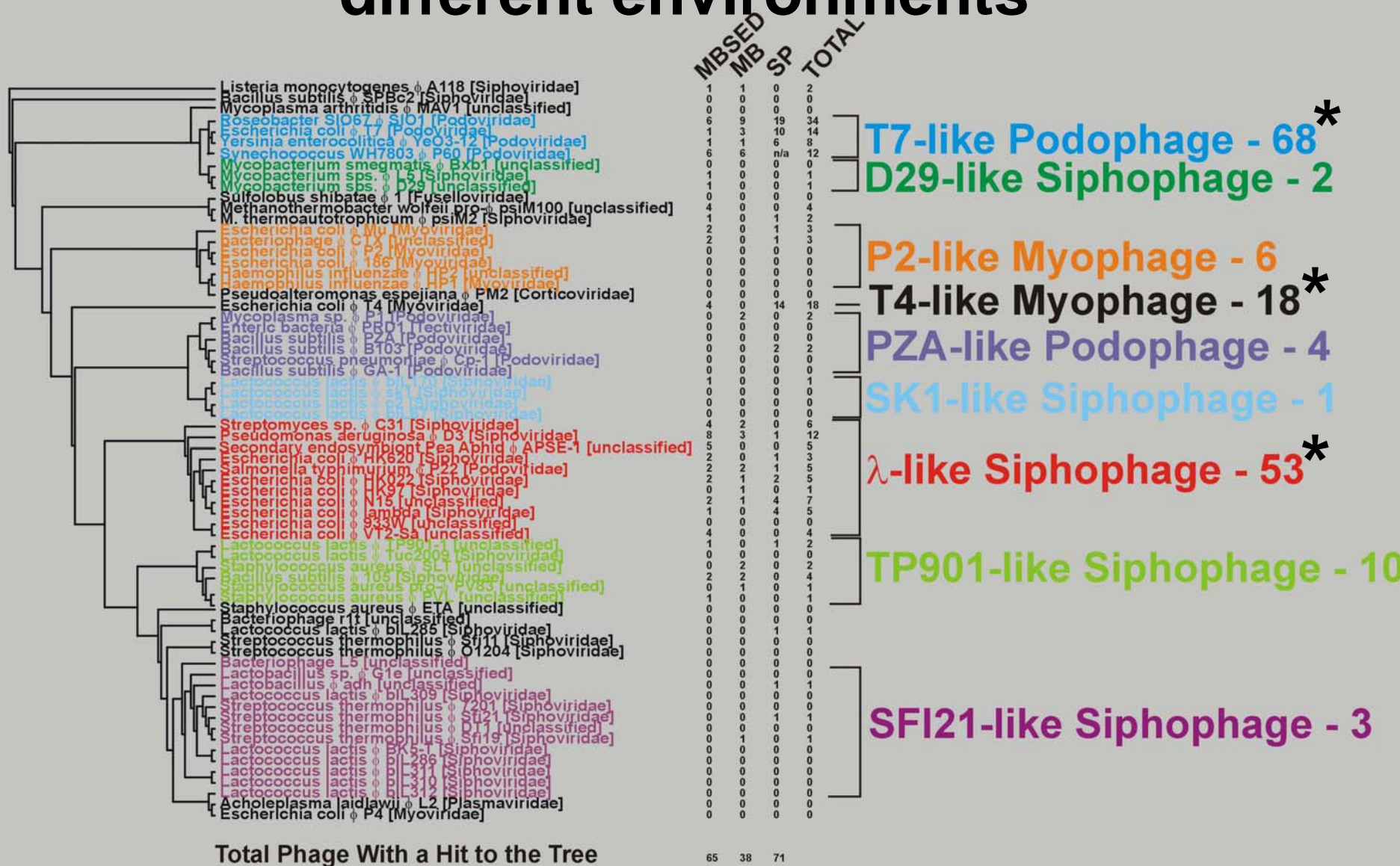


~95% chance of placing fragment in "correct" group
 "metagenomic species"

Edwards & Rohwer (2005) Viral Metagenomics. Nature Reviews in Microbiology. 3 (6). 504-510.

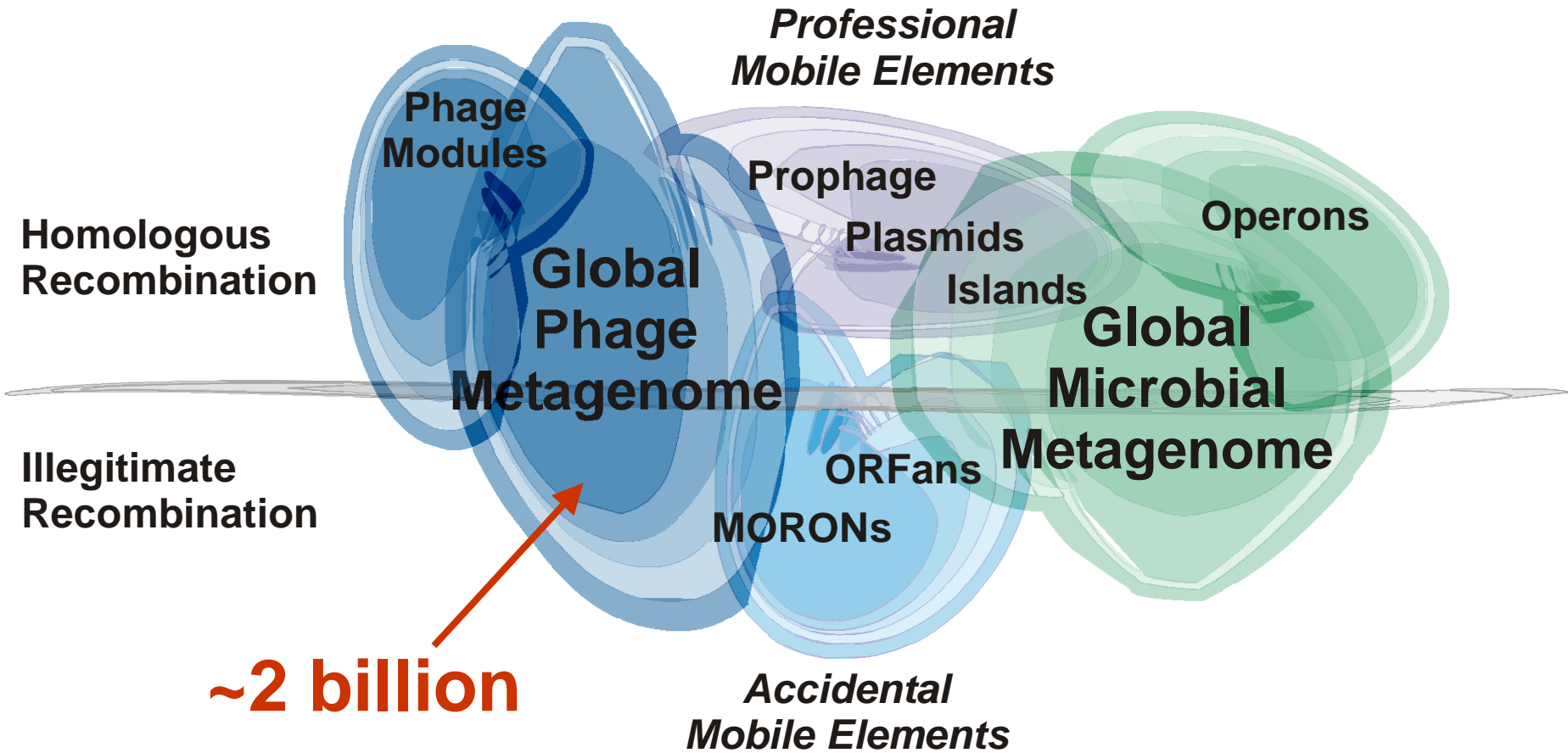
Rohwer & Edwards (2002) "The Phage Proteomic Tree..." J Bact. 184 (16). 4529.

Distinct phage groups dominate in different environments



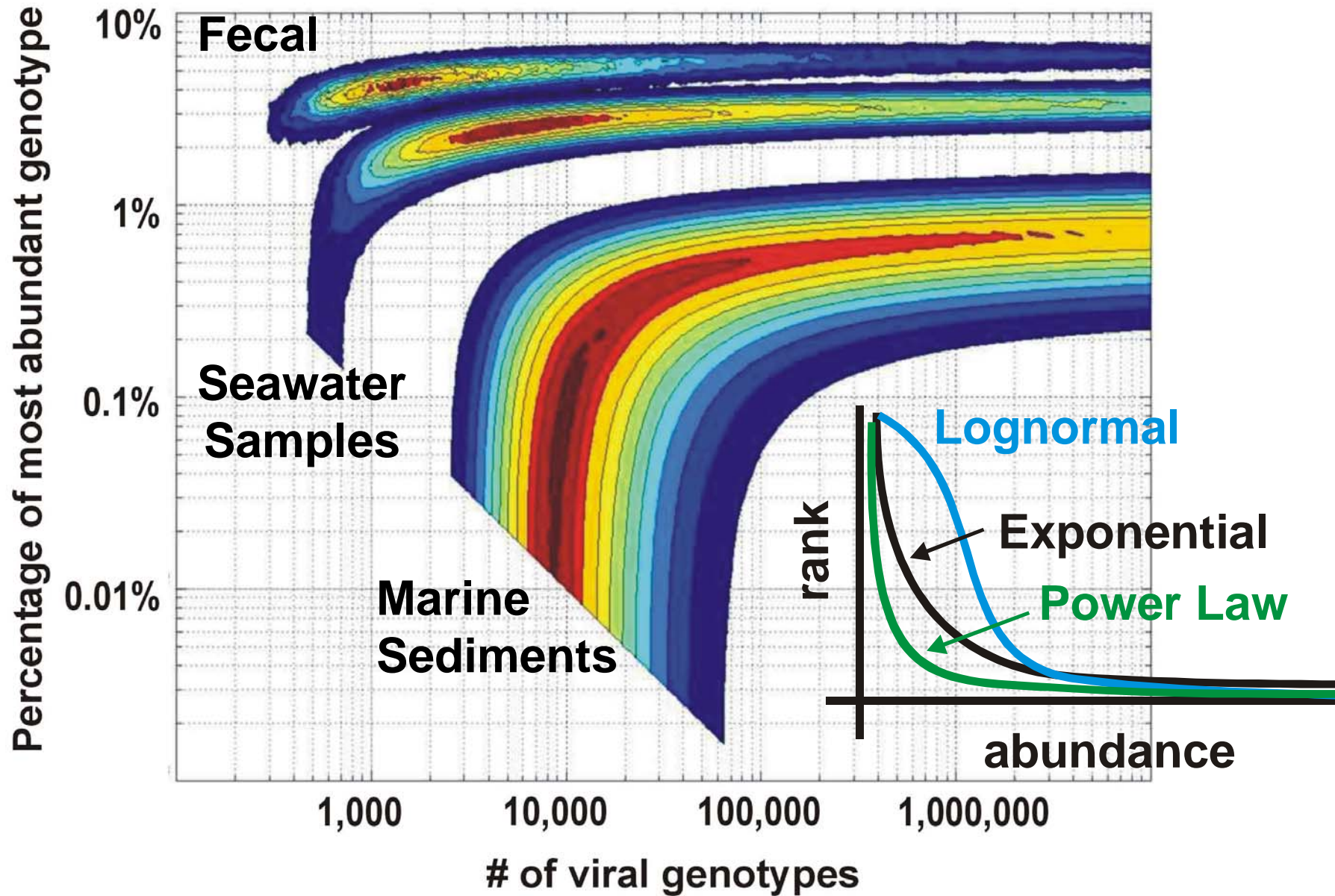
Metagenomic “signatures” of different environments

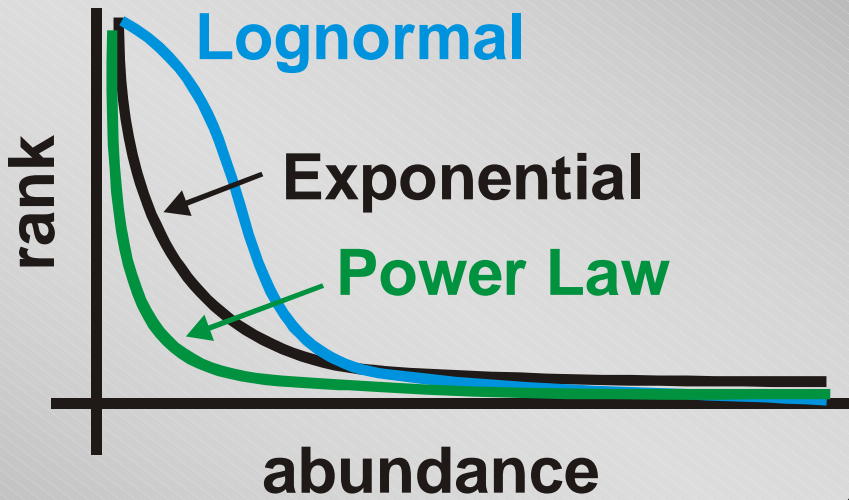
Quantum landscape of genes



Breitbart & Rohwer (2005) Here a Virus, There a Virus, Everywhere the Same Virus?.
Trend in Microbiology. 13 (6). 278-284.

Viral community structure





Determine the actual contig spectrum of the sample



Predict a contig spectrum using a species abundance model

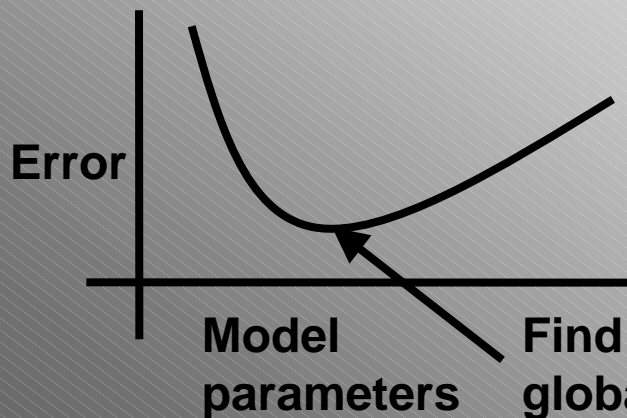


Compute the error between the actual and predicted



Adjust the parameters in the species abundance model to minimize errors

Continue this procedure to obtain the smallest error



PHACCS - Phage Communities from Contig Spectrum - Mozilla Firefox

File Edit View Go Bookmarks Tools Help

http://phage.sdsu.edu/phaccs/

BioMed E-Journals - H... ISI Web of Knowledg... Google Scholar

Bookmarks

Search:

Bookmarks Toolbar... BioDiversity Analys... Cool Science Cool Marine Illus... Corals Fishing Genomics Global Cell Number http--research.hq... http--www.sci.sdsu... Lab Website Coral Disease E... eXTRemE Track... FastGroupII Ho... Lab Homepage LASLs Homepage VVictors Vibrioph... Every BLAST se... PHACCS Rohwer Lab Libraries BIOSIS Search ... Current Content... Library & Infor... SIO E-Journals UCSD Libraries ELECTRONIC J... NIH Maps World Atlas Outi... NASA World Wind jules homepage... NSF Phage Phage Math Page Photography Pollstar -- The Con... Ribosomal RNAs Robs scripts SDSU Foundation PI Profile Login ...

PHACCS
Phage Communities from Contig Spectrum

[Home](#) | [Contig spectrum analysis](#) | [Resource](#) | [Program](#) | [Contact](#)

Contig spectrum analysis

Advanced interface

The advanced interface is for the [custom analysis](#) of any [viral community](#) and predictions about its:

- **structure**: best relative abundance functional form and model's equation, and
- **diversity**: richness, evenness, Shannon-Wiener index, relative abundance of the most abundant genotype.


> Data

- Contig spectrum: ?
- Avg. genome size (bp): ?
- Avg. fragment length (bp): ?
- Min. overlap length (bp): ?

> Computation


- Rank-abundance distribution:
 - Power Exponential
 - Logarithmic Lognormal
 - Niche Preemption Broken Stick
 -
- Genotype range: from to ?
- Precision: ?
- Graphics:
 - Error curve ?
 - Abundance curve
 - Abundance curve (log scale)

Note: Depending on your analysis the computation can take a while. Please be patient!

 [Switch to the basic interface if you don't know what to put in these fields.](#)

For questions, suggestions or bug reports, please contact the webmaster.

Done

Start |  | 10:39 AM

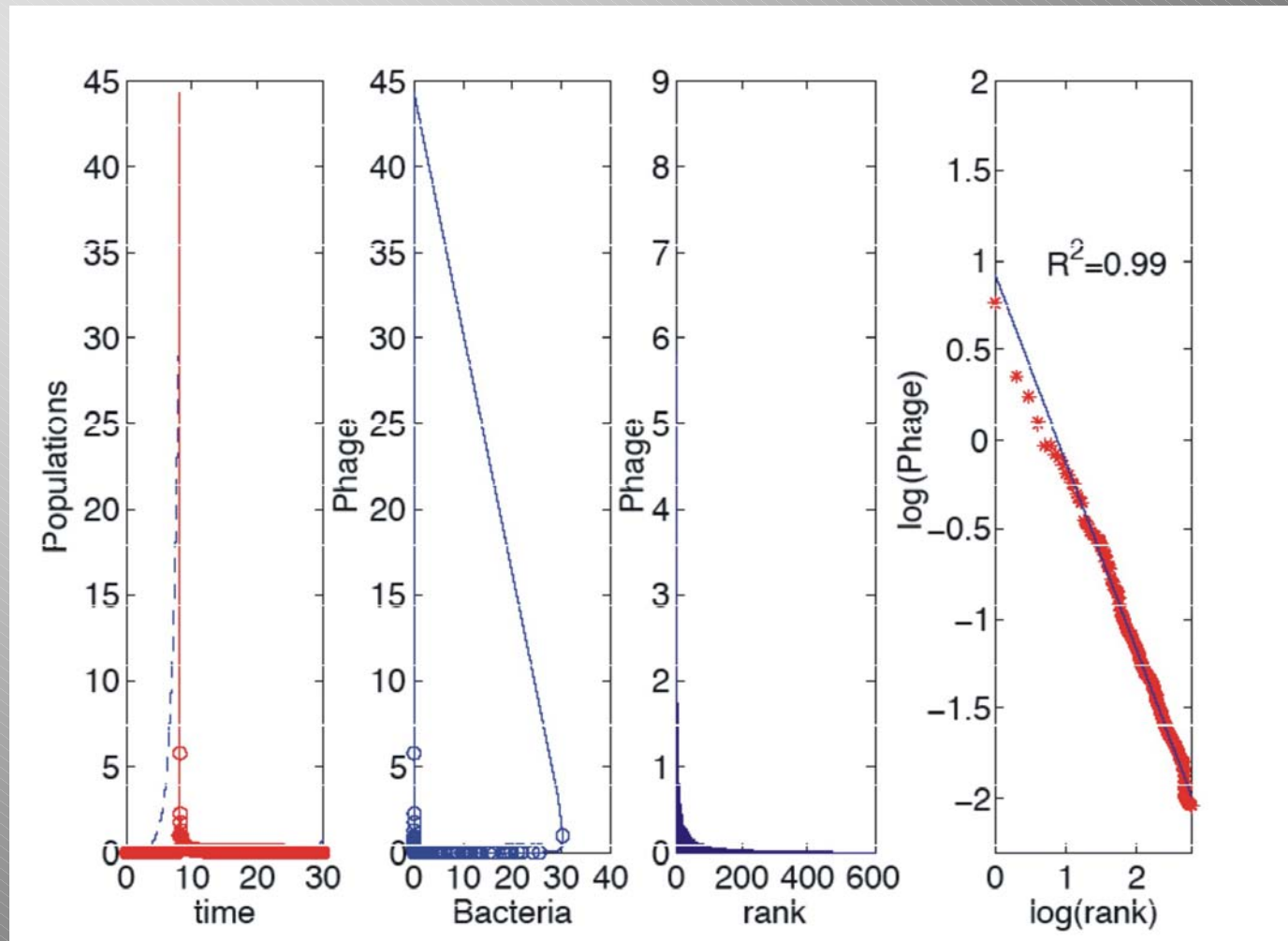
Angly et al. (2005) PHACCS, an online tool for estimating the structure and diversity of uncultured viral communities using metagenomic information. *BMC Bioinformatics*. 6:41

Power Law is the best explanation (so far)

Model	% most abundant	Error	Number of species	Parameters of the model
Power Law	2.65	2.11	7420	B=0.73062
Exponential Law	0.42	16.2	7500	B=0.0042156
Logarithmic	3.02	2.81	1560	B=1.8054
Broken Stick	0.78	14.6	960	No additional parameters
Niche Preemption	0.476	38.1	8200	K=0.0047582
Lognormal	2.77	2.31	43110	Sigma=2.2237

Fewer "species"
Models of community structure

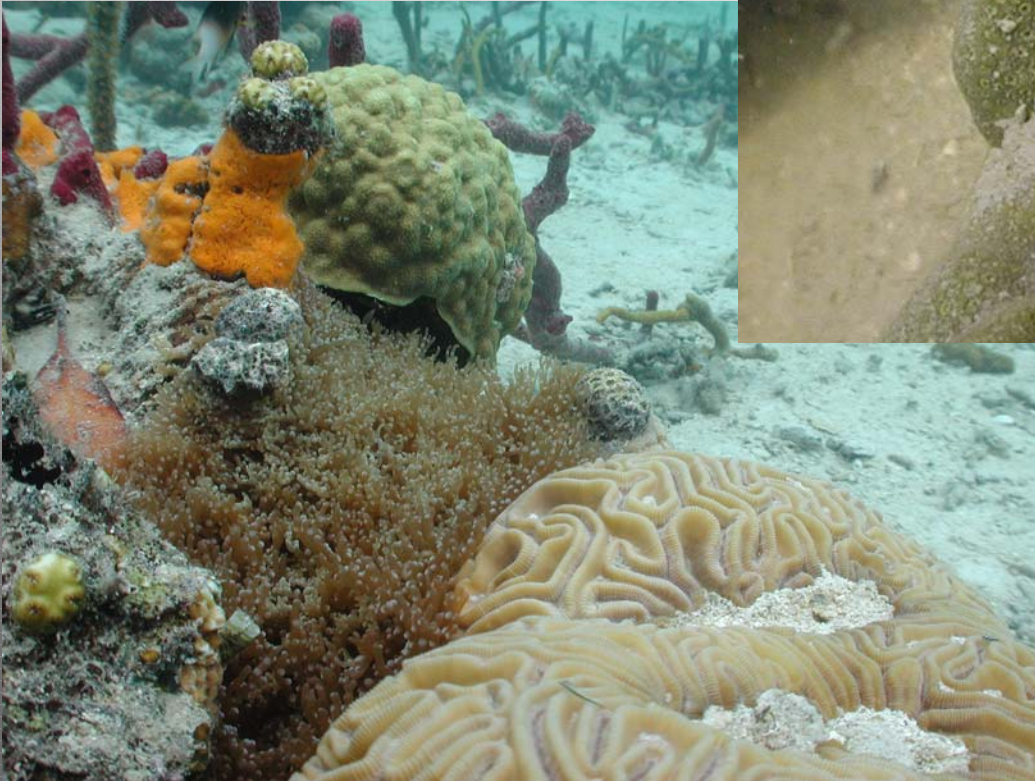
Generalized Lotka-Volterra



"Kill-the-Winner"

Corals are heavily colonized by prokaryotes

$\sim 10^7$ - 10^8 prokaryotes
per cm^2



Wegley, et al. 2004. Coral-associated Archaea. MEPS. 273. 89-96.

***Diploria strigosa*, *Porites astreoides*, &
Montastraea franksi from Bermuda and
Panama (2 different years)**

Isolate total DNA



PCR amplify 16S rDNAs from Bacteria



Make library



Sequence 5' 550 bp from ~ 100 clones per sample

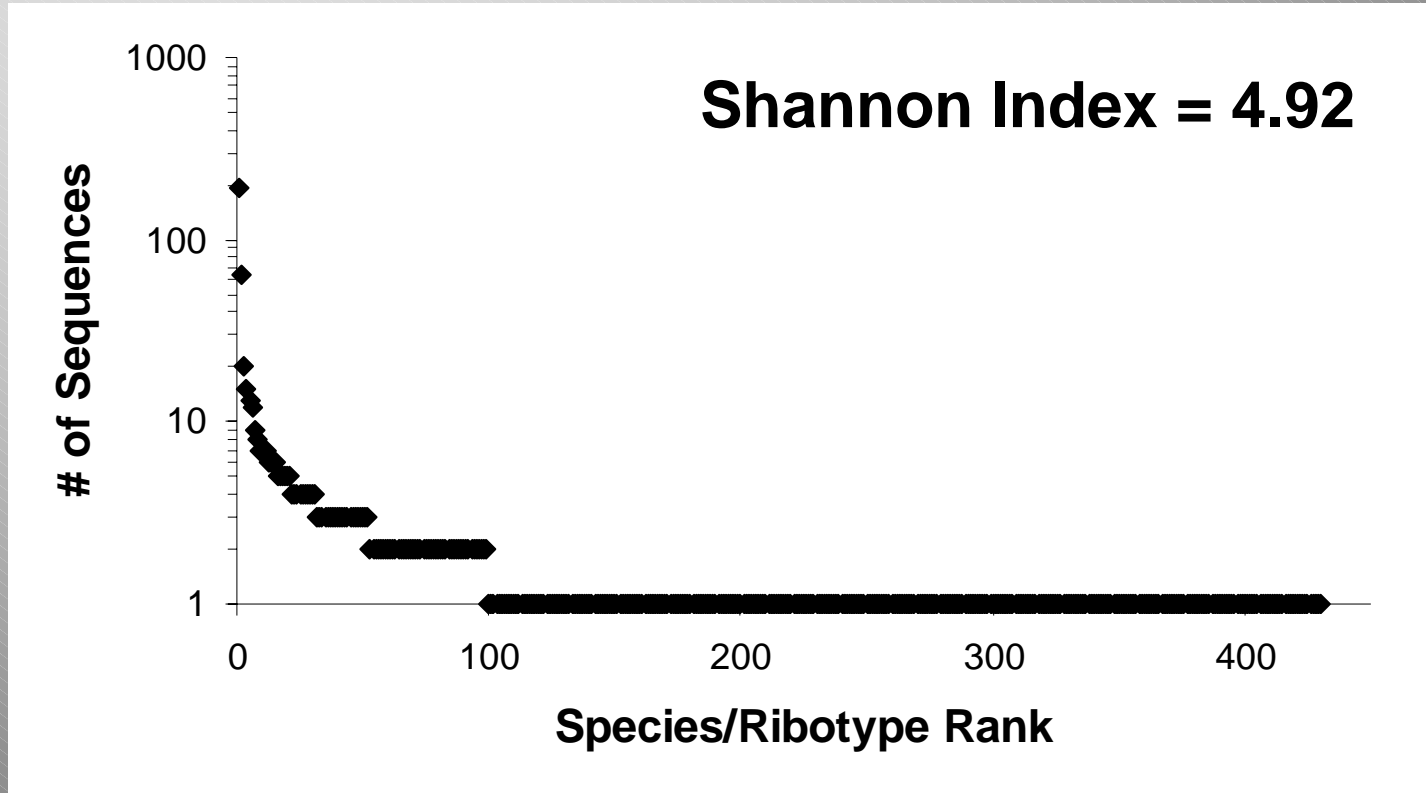


Analyze with FastGroup, ClustalW, and BLAST

- total of 1,178 rDNA seqs from 14 samples

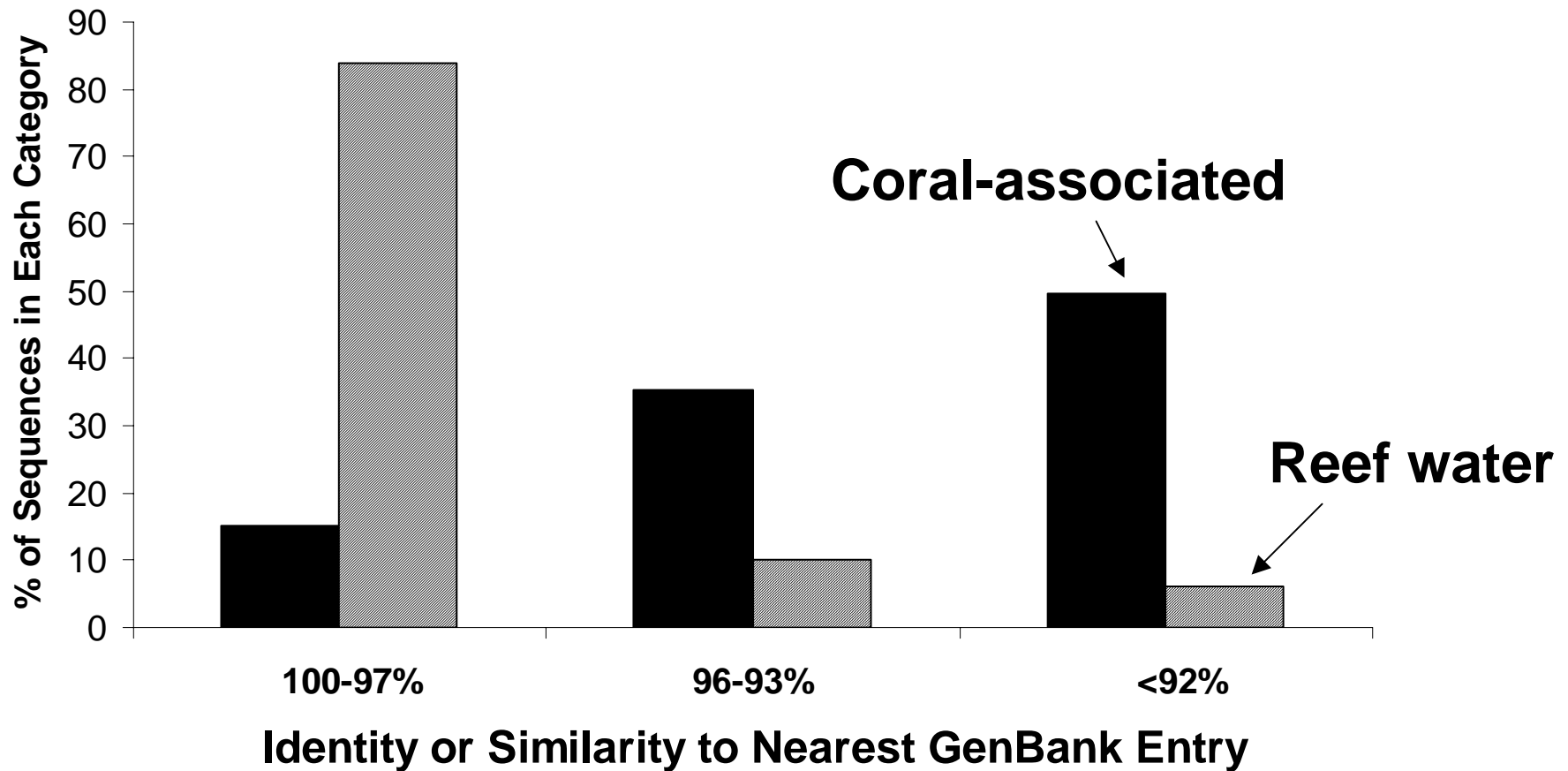
**Consider sequences with $\geq 97\%$ identity as
the same ribotype**

Diversity of coral-associated bacteria is very high



- 430 distinct bacterial ribotypes from 14 corals
- Chao2 predicts 6,000 ribotypes

Most coral-associated bacteria are novel genera &/or species



Corals located next to each other have unique microbiotas



P. astreoides



M. franksi

D. strigosa



Three corals from Bermuda shared 1 out of 101 ribotypes
Three corals from Panama shared 0 out of 123 ribotypes

Coral-bacteria associations are maintained over time and distance

	<i>Porites astreoides</i>					<i>Diploria strigosa</i>				<i>Montastraea franksi</i>					AVE
	BER1	BER2	PAN4	PAN5	PAN5	BER1	BER2	PAN4	PAN5	BER1	BER2	BER2	PAN2	PAN5	
Alpha	9	1	9	0	10	7	11	21	26	25	34	15	19	47	17
Gamma	13	5	2	14	22	13	15	26	18	20	12	35	15	20	16
PA1 (Gamma)	40	92	72	77	27	0	0	0	0	0	0	0	0	0	22
CFB	13	0	0	0	7	43	28	17	26	8	3	6	19	9	13
BC	0	0	0	7	15	32	24	35	26	12	4	11	4	11	13
Cyano	6	0	17	2	8	0	12	0	0	10	4	2	7	10	6
Other	18	2	1	0	11	5	10	3	3	25	42	31	37	2	14
n	61	143	88	54	73	75	126	81	81	87	82	70	62	95	

- PA1 is a γ -proteobacteria always associated with *P. astreoides*
- Cytophaga-Flexibacter-Bacteroides (CFB) and Bacillus-Clostridium (BC) groups are preferentially associated with *D. strigosa*
- Diversity is higher in Panamanian samples, which parallels metazoan biodiversity gradients

Conclusions

- The coral holobiont harbors all three Domains of life
- Different coral species have unique bacterial populations
- Specific bacteria-coral associations are maintained over geographic and temporal space
- Individual coral colonies have microniches that harbor distinct bacteria
- For every coral colony:

1 coral animal:2-3 zoox species:60-? prokaryotic species

Rohwer Lab - SDSU

**Mya Breitbart
Beltran Rodriguez-Brito
Pat McNairnie
Florent Angly
Linda Wegley
Veronica Casas
Jon Miyake**

Rob Edwards - SDSU

Chisholm Lab - MIT

Matt Sullivan

Math Guys - SDSU

**Peter Salamon
Joe Mahaffy
Bjarne Andresen
James Nulton
Ben Felts
David Bangor**

Anca Segall - SDSU

David Mead - Lucigen

John Paul - USF

**The Gordon and Betty Moore Foundation
- Marine Microbiology Initiative**

**NSF - Biotic Surveys and Inventories
- Biological Oceanography
- Biocomplexity**

