Trends in *Lophelia* Microbiology: From the Gulf to Global

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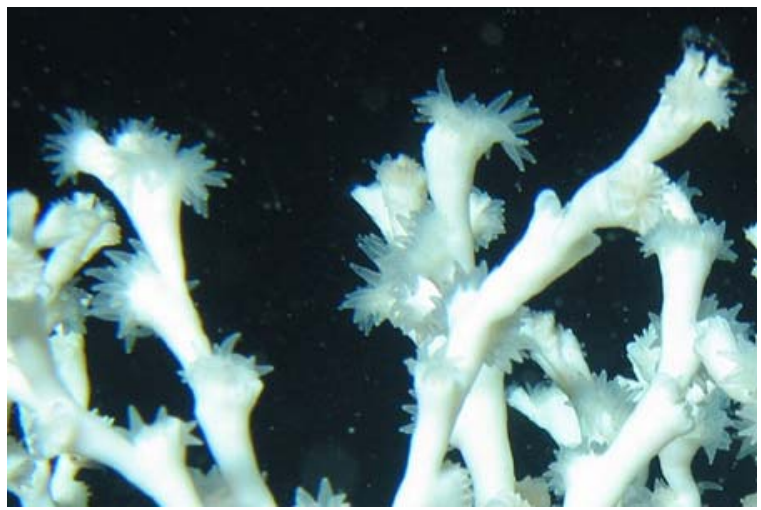
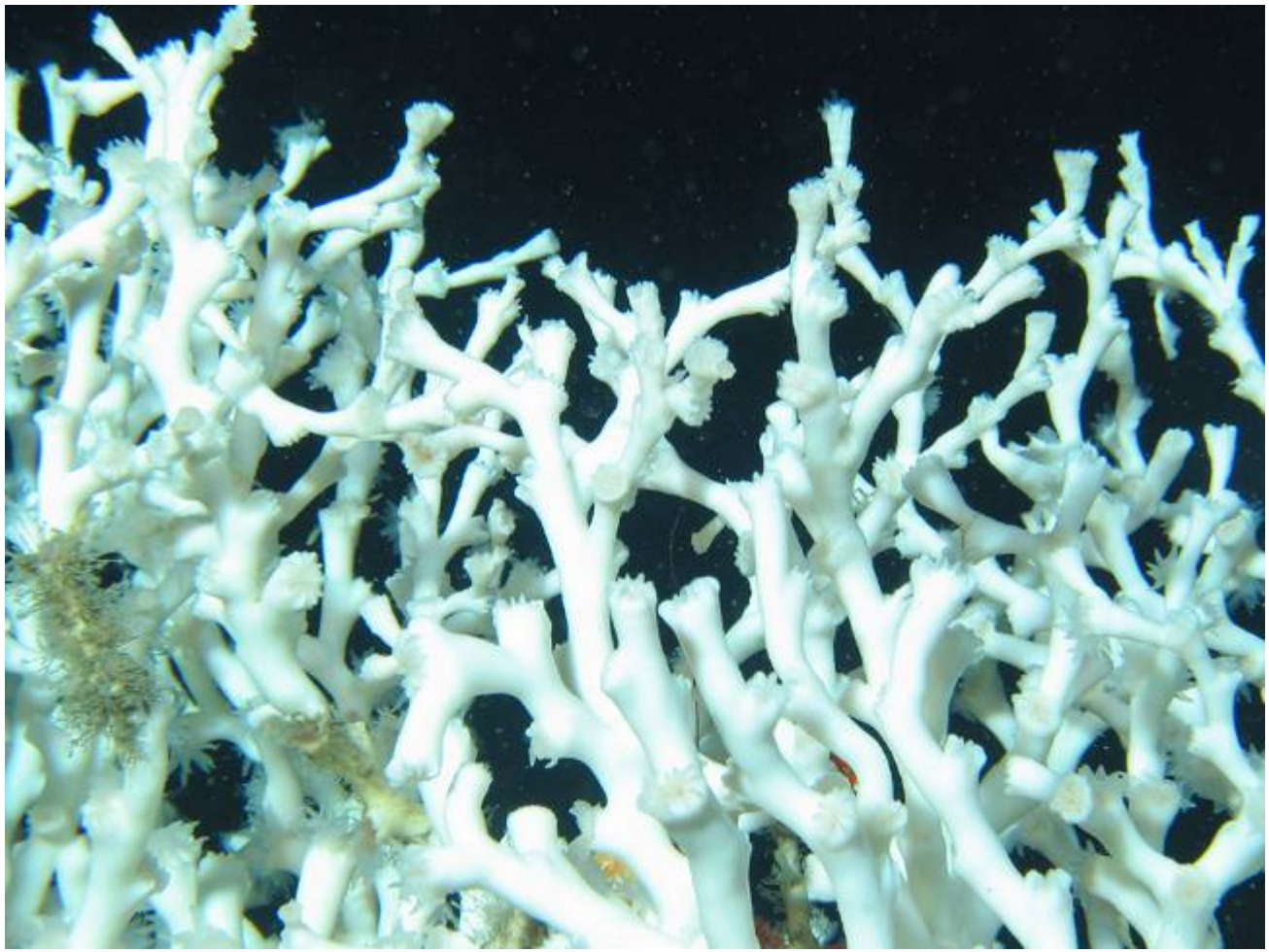


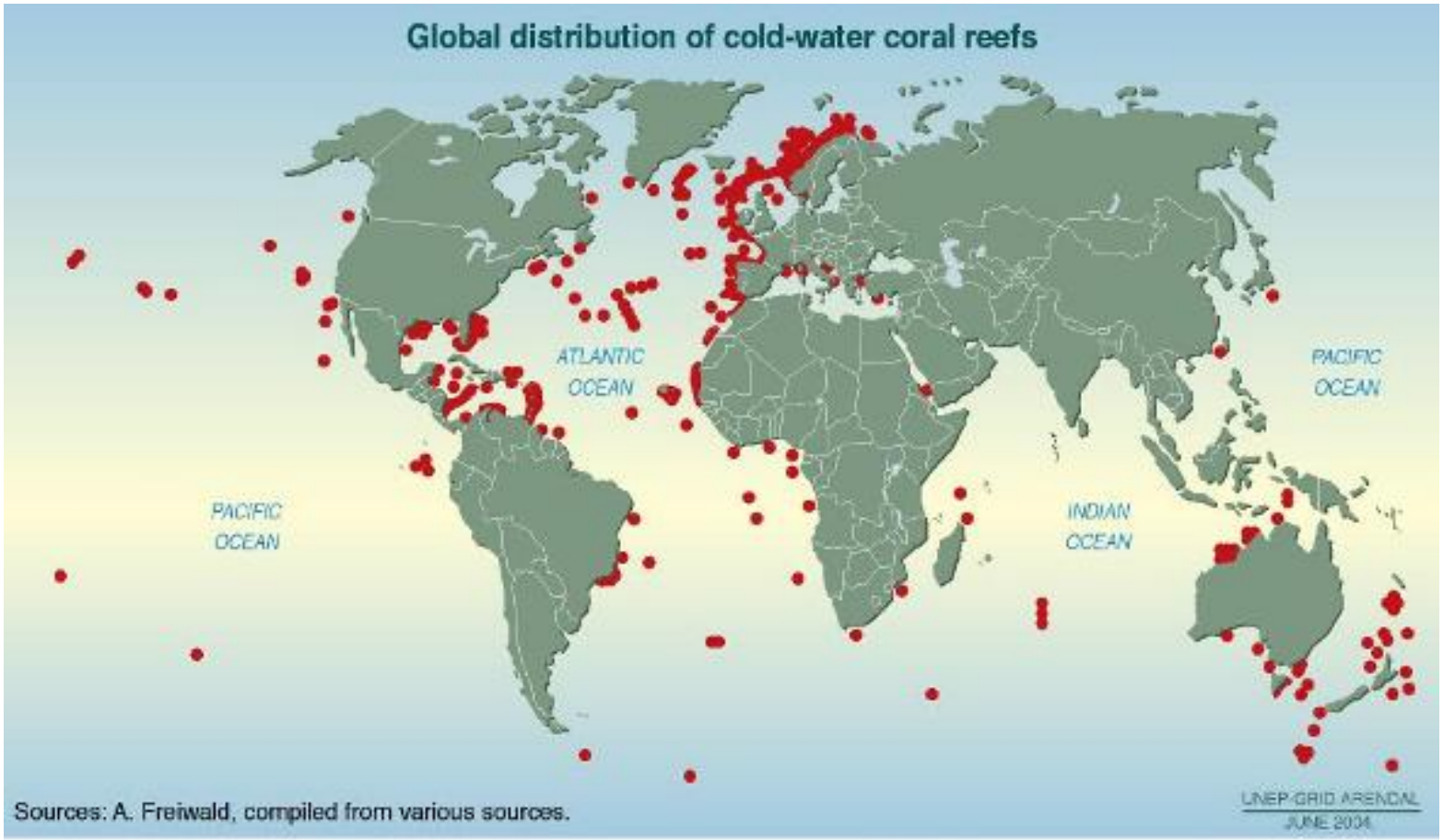
MMS Information Transfer Meeting, January 2009

Lophelia pertusa



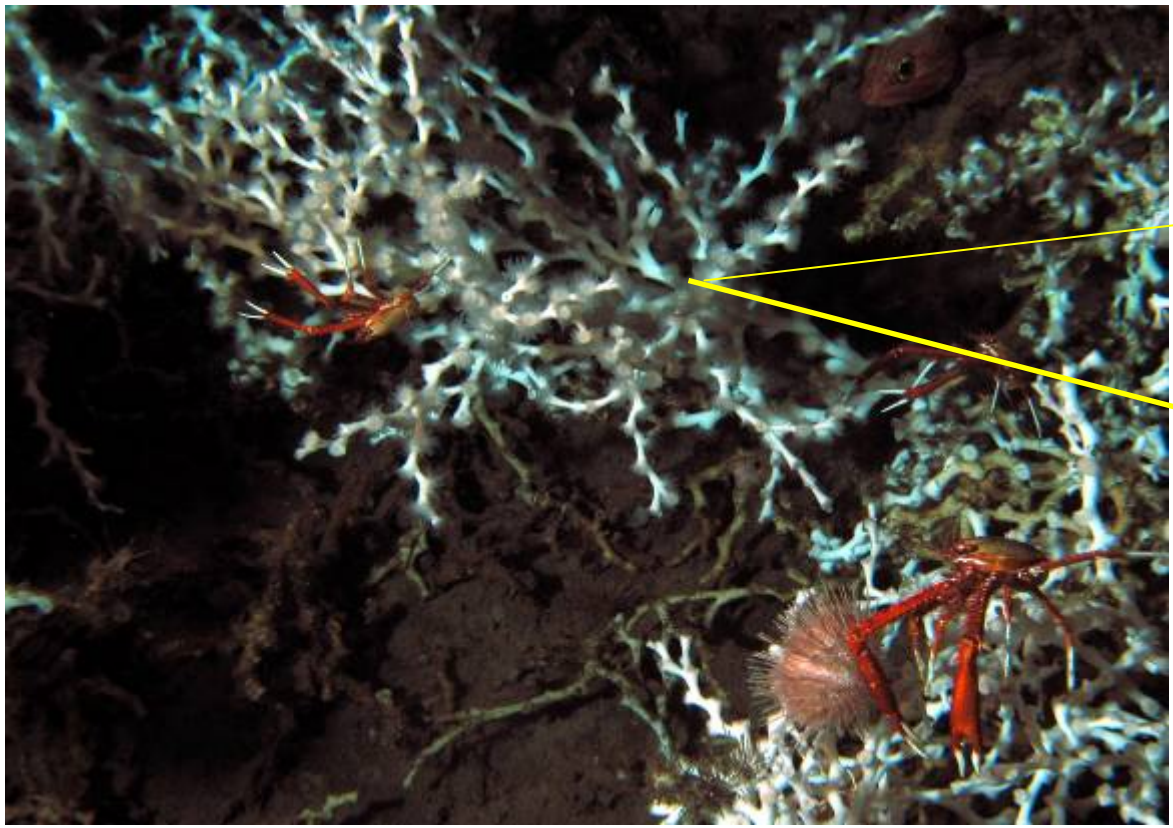
USGS

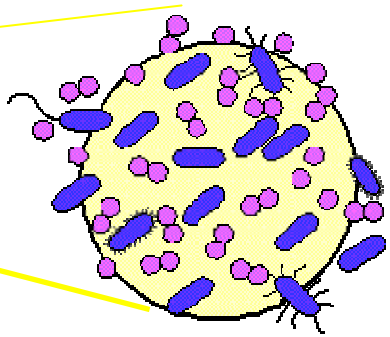
Distribution



Global distribution of cold-water coral reefs: points on the map indicate observed reefs of varying size and stages of development but not the actual area covered. The high density of reefs shown in the North Atlantic reflects the intensity of research in this region. Further discoveries are expected worldwide, particularly in the deeper waters of subtropical and tropical regions.

Why do the microbes matter?





Coral Microbial Ecology

Coral = animal + algae + bacteria + archaea + fungi + viruses

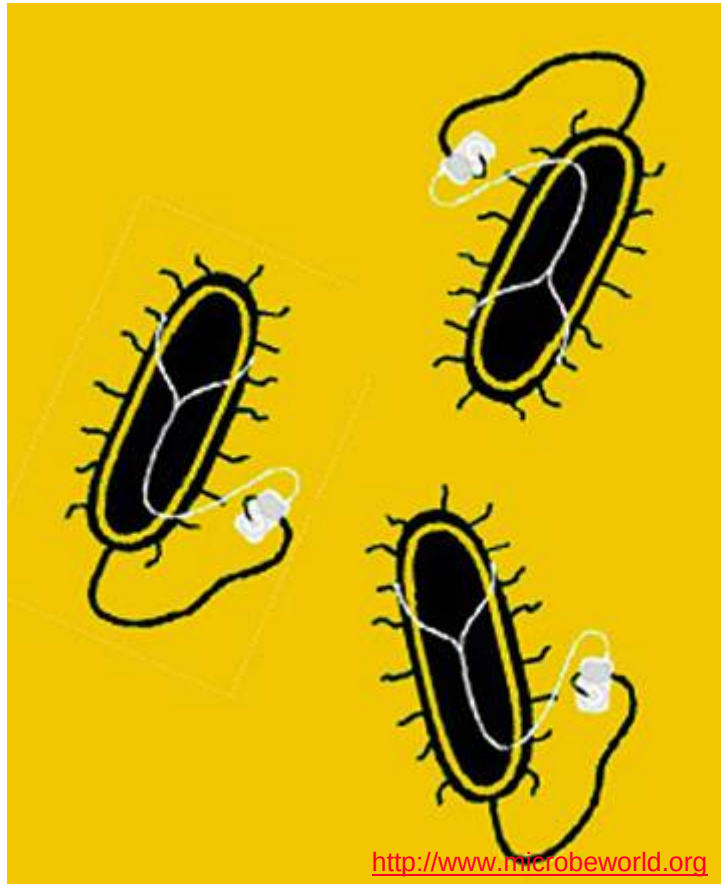
Coral = animal + microbiome

Where are they?

- Coral surface microlayer
- Tissue
- Skeleton

What are they doing?

- Being dinner?

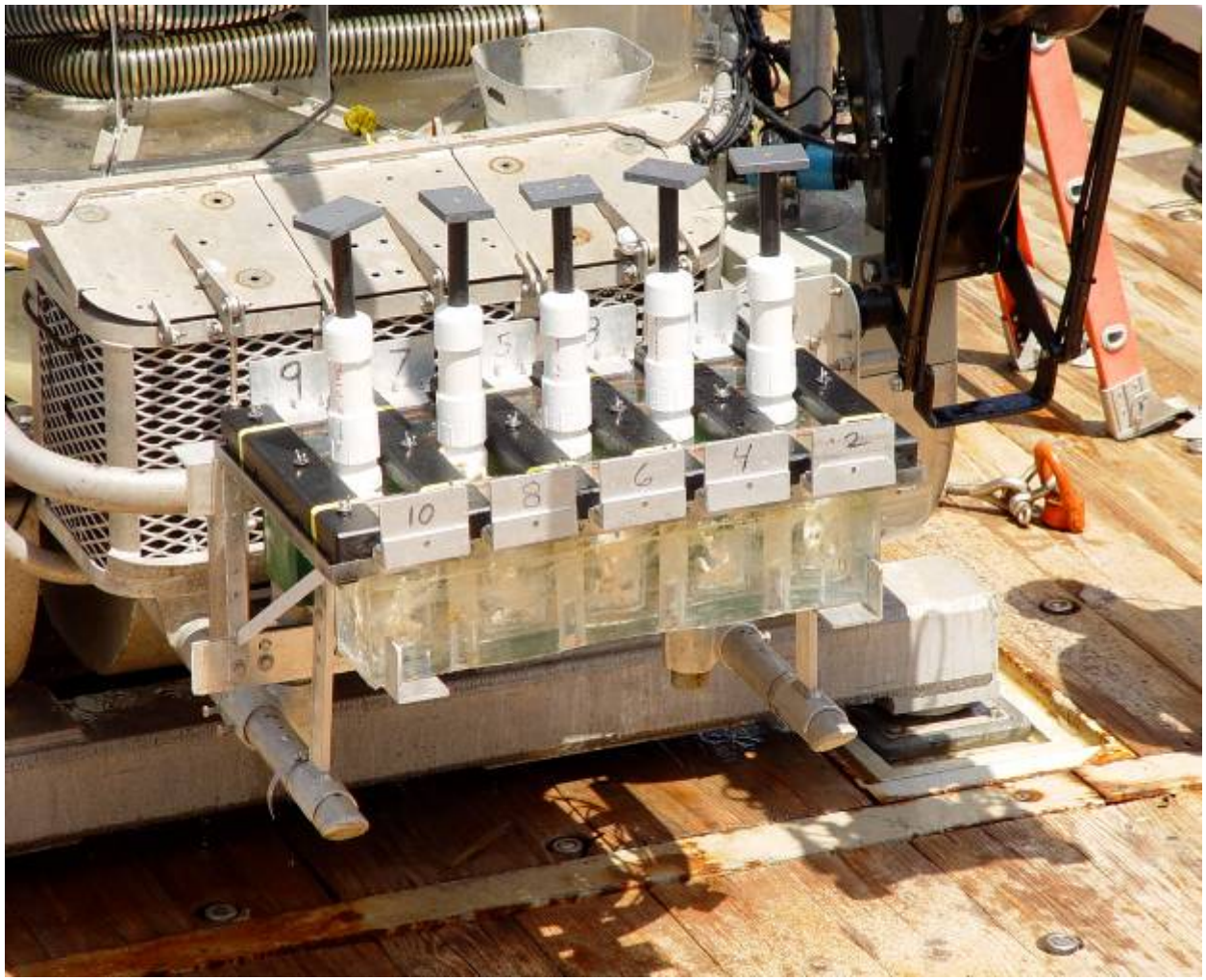


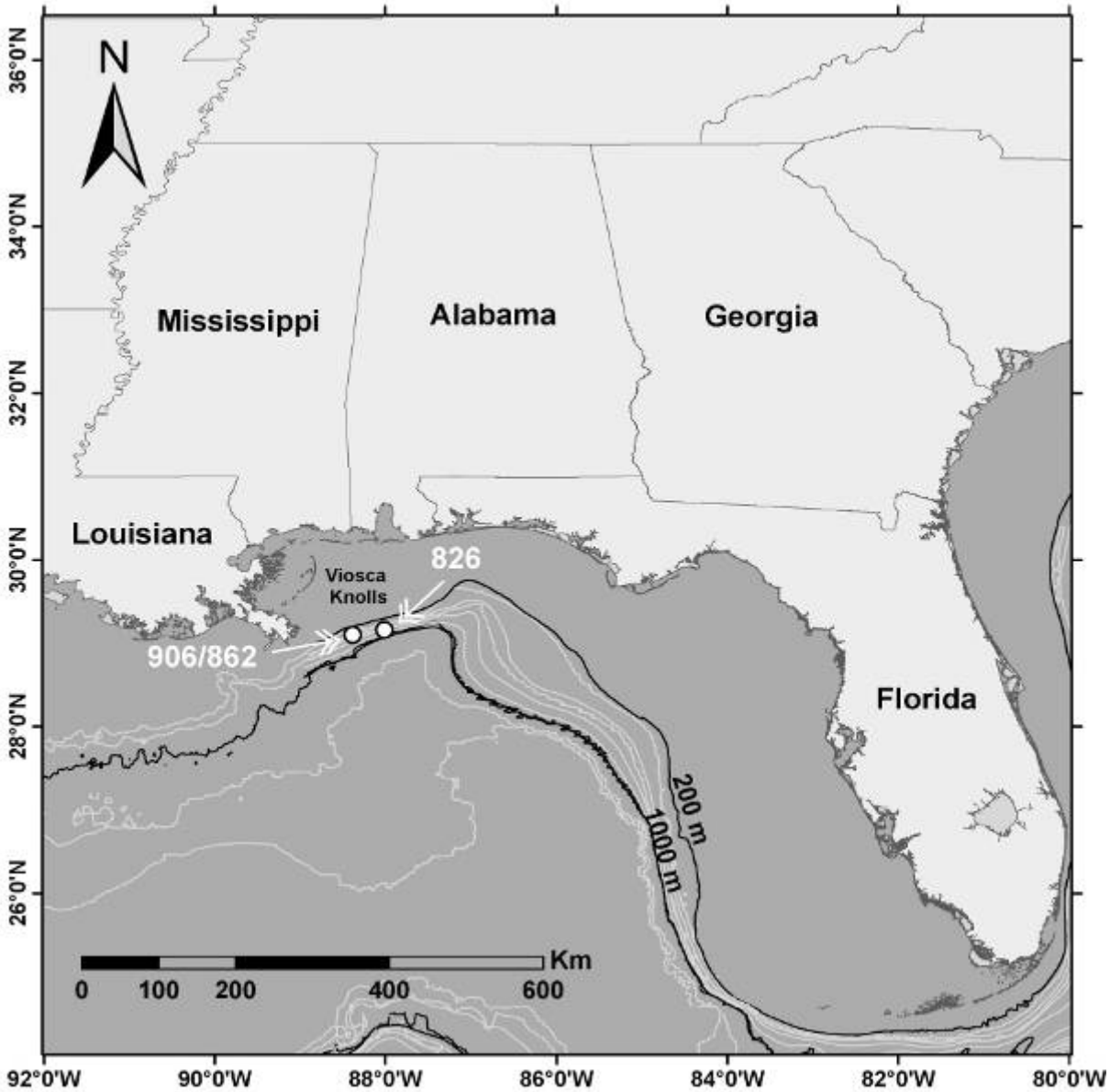
- Fixing nitrogen
- Making antibiotics
- Occupying niches
- Gathering iron, phosphorus
- Cycling metabolic waste compounds

Sampling Concerns

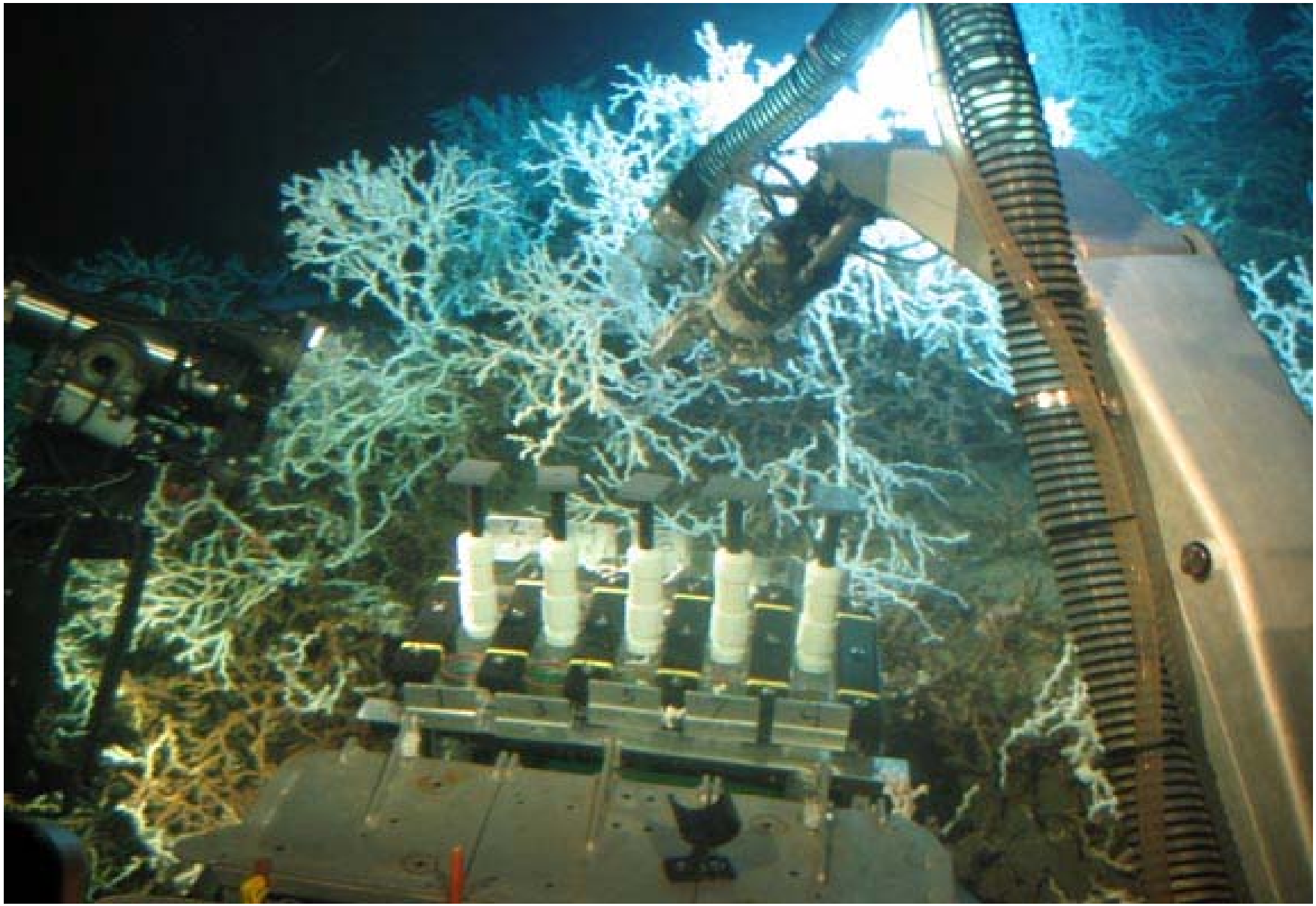
- **Contact issues – water column, sediment, other invertebrates**
- **Taking samples through an environmental gradient of pressure, temperature, and light**

‘The Acrylic Beast’




N
Mississippi
Alabama
Georgia
Louisiana
Florida
Viosca
Knolls
826
906/862
200 m
1000 m
Km
0
100
200
400
600
36°0'N
34°0'N
32°0'N
30°0'N
28°0'N
26°0'N
92°0'W
90°0'W
88°0'W
86°0'W
84°0'W
82°0'W
80°0'W


HARBOR BRANCH



Methods

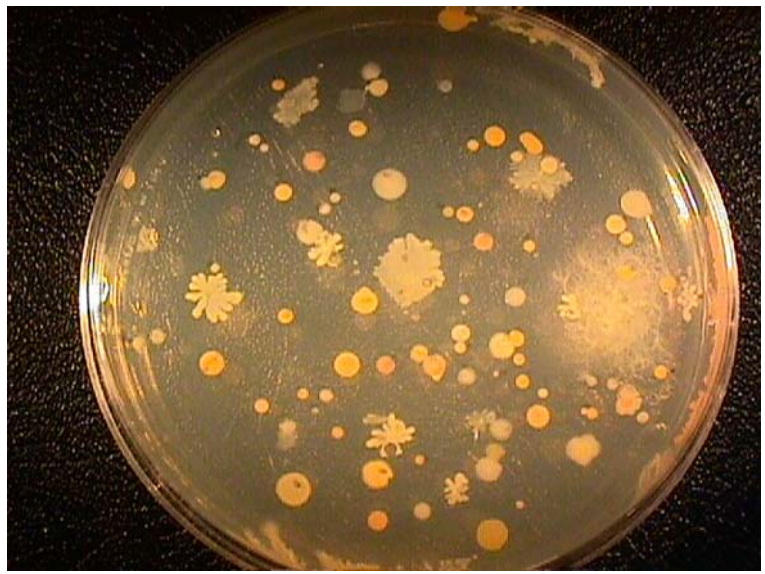
- Samples = mucus/tissue/skeleton
- Culture on marine agar – non-preserved samples
- Extract total DNA – preserved samples
- Amplify DNA using PCR + specific primers, clone, sequence

Lophelia Culture Data

- ***Achromobacter* sp. (98%)**
- ***Alteromonas nigrifaciens* (98%)**
- ***Photobacterium* spp. (99%)**
- ***Pseudoalteromonas* spp. (98%)**
- ***Pseudomonas stutzeri* (99%)**
- ***Psychrobacter nivimaris* (100%)**
- **7 *Vibrio* spp. (98-99%)**

Lophelia Culture Data

- ***Cobetia* spp. (96–100%)**
- ***Colwellia* sp. (93%)**
- ***Halomonas* sp. (97–99%)**
- ***Moritella* sp. (94%)**
- ***Shewanella* spp. (96–98%)**



Lophelia Culture Data

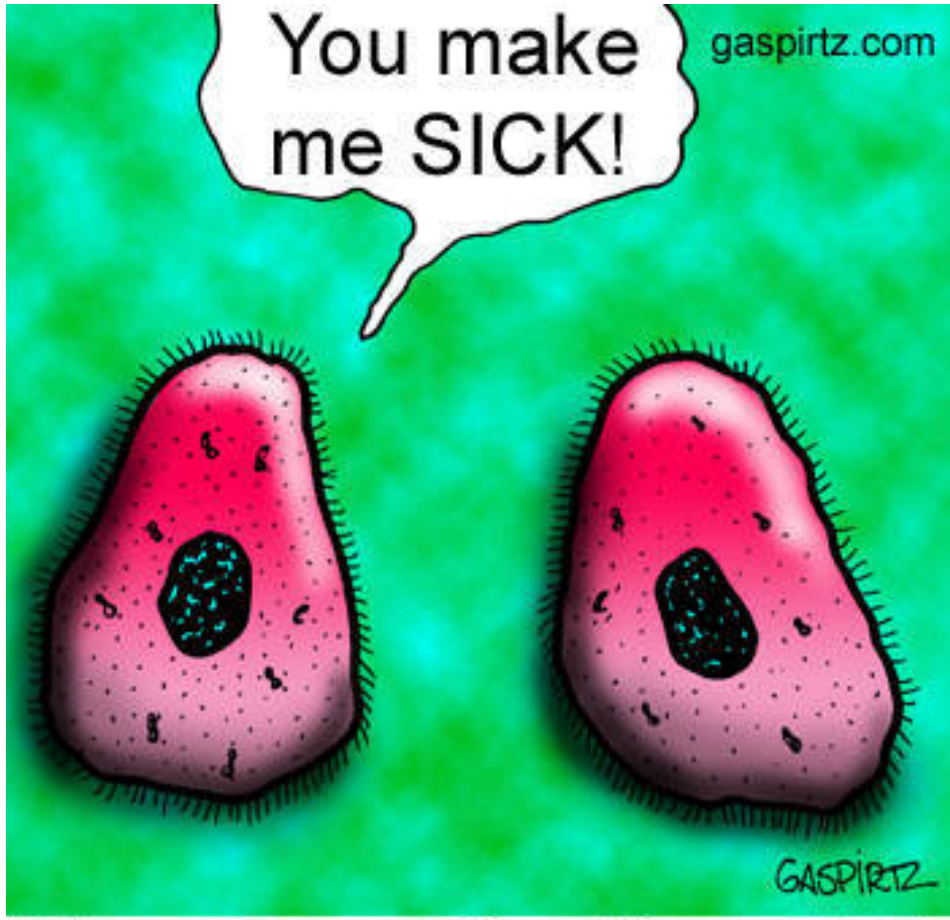
- **Previously uncultured bacterial clone 131851 (99%) – from whale bone at 560 m**

- **Unknown *Alteromonadaceae* (85–90%)**
- **Unknown *Tenerictues (mesoplasma-like)* (89%)**
- **Unknown *Vibrionaceae* (82%)**

Lophelia Culture Data

- **Modified Kirby-Bauer antibiotic susceptibility testing in progress**

- **So far (23 of 213), there is uniform resistance to penicillin and clindamycin. Variable responses to tetracycline, polymixin B, chloramphenicol and novobiocin.**



When germ relationships go bad

Lophelia – 16S Clone Libraries

- **Seven clone libraries, 508 total clones analyzed**
- **Three *Lophelia* colonies from VK906/862, three from VK826**
- **93% clones were novel (<97% similarity)**

Lophelia – 16S Clone Libraries

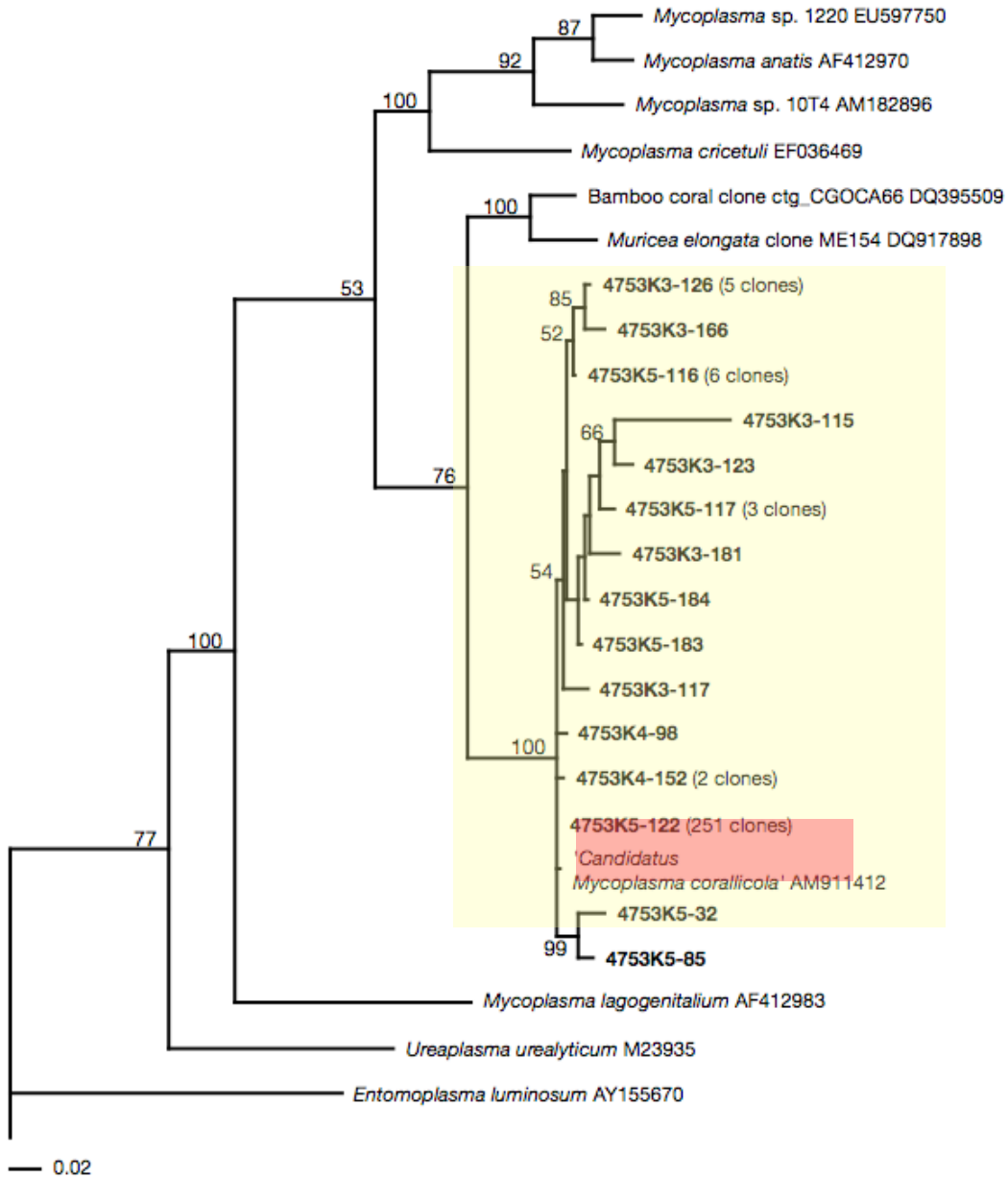
- **52% were unknown *Tenericutes* (mycoplasmas)**
  - **Mycoplasmas lack cell walls**
  - **Many are pathogens or obligate cellular parasites**
  - **Associated with deep-sea bamboo corals [Penn et al. 2006]**
  - **Found in one bleached shallow-water coral [GenBank]**


Photo: Cheryl Morrison

Lophelia – 16S Clone Libraries

- **Global picture**
  - **Mediterranean *Lophelia* – no mycoplasmas [Yakimov et al. 2006]**
  - **Norwegian fjord *Lophelia* – plenty of mycoplasmas! [Neulinger et al. 2008]**


Photo: Cheryl Morrison


Mycoplasma sp. 1220 EU597750
Mycoplasma anatis AF412970
Mycoplasma sp. 10T4 AM182896
Mycoplasma cricetuli EF036469
Bamboo coral clone ctg_CGOCA66 DQ395509
Muricea elongata clone ME154 DQ917898
4753K3-126 (5 clones)
4753K3-166
4753K5-116 (6 clones)
4753K3-115
4753K3-123
4753K5-117 (3 clones)
4753K3-181
4753K5-184
4753K5-183
4753K3-117
4753K4-98
4753K4-152 (2 clones)
4753K5-122 (251 clones)
'Candidatus Mycoplasma corallicola' AM911412
4753K5-32
4753K5-85
Mycoplasma lagogenitalium AF412983
Ureaplasma urealyticum M23935
Entomoplasma luminosum AY155670
87
92
100
100
53
85
52
66
76
54
100
100
77
99
0.02

Lophelia – 16S Clone Libraries

- **33% were gamma-proteobacteria**
- **9% were alpha-proteobacteria**
- **31% were most similar to other coral-associated seq**
- **24% were related to thiotrophic bacterial symbionts of cold-seep clams**

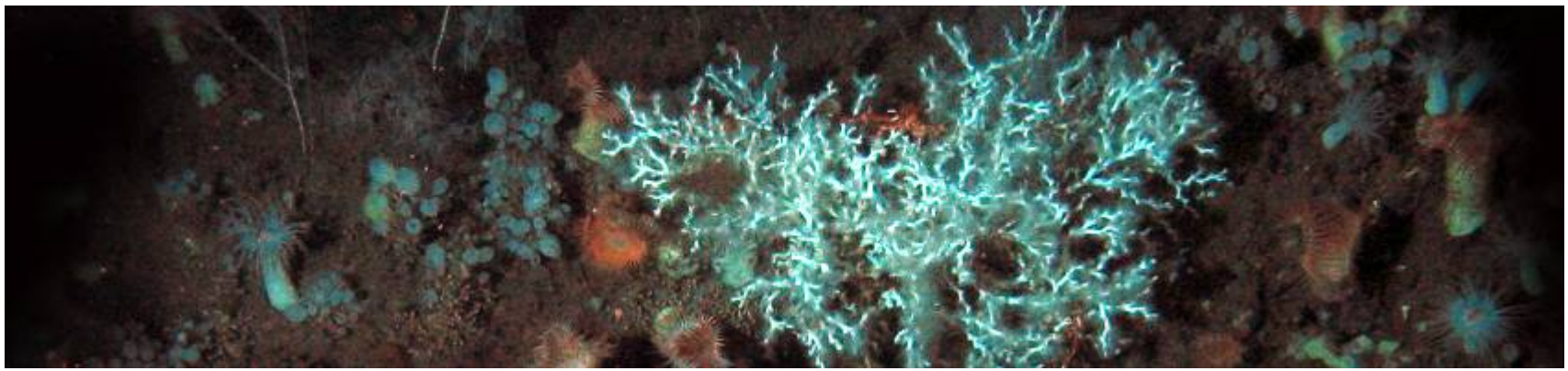
Lophelia – 16S Clone Libraries

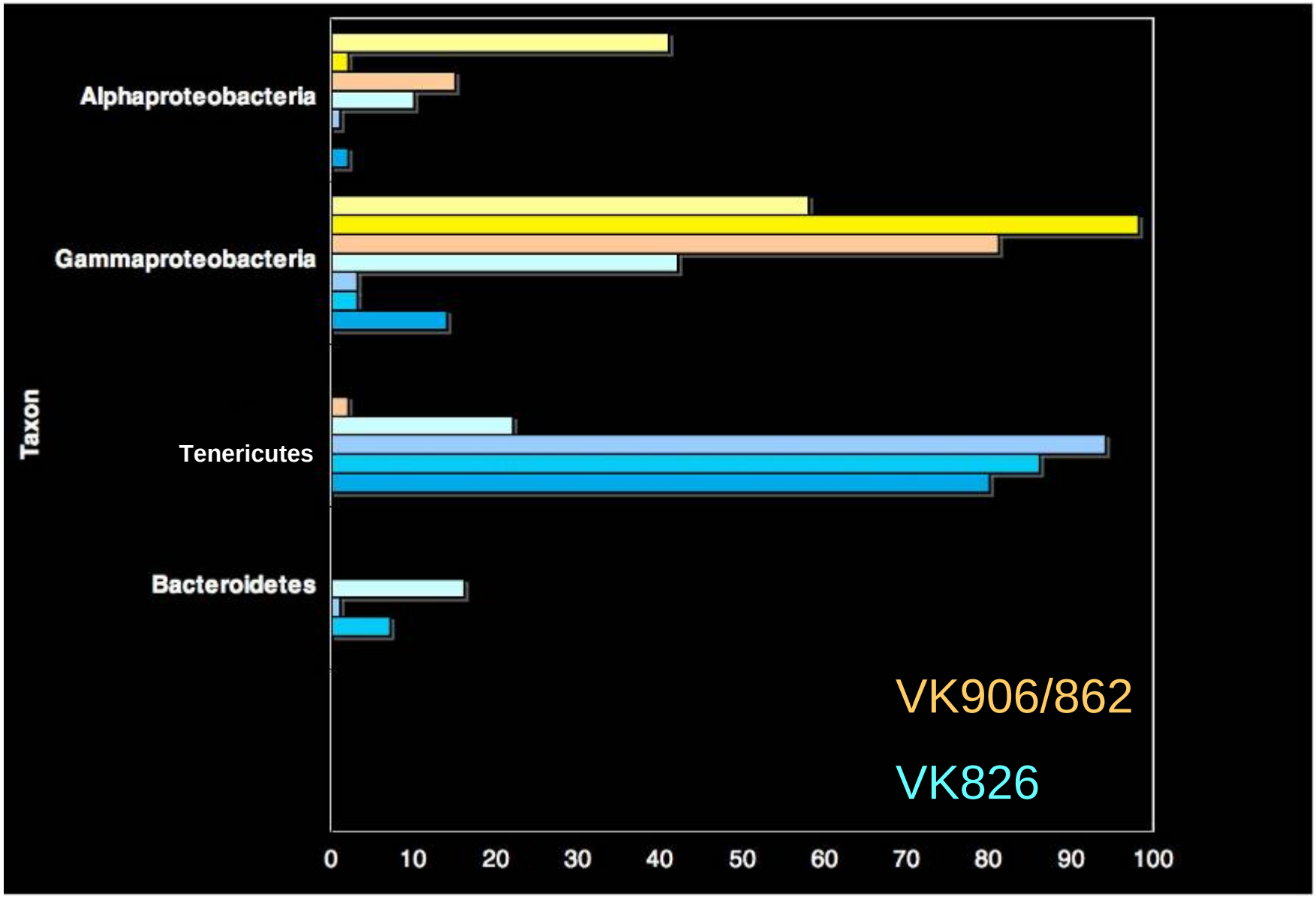
- **Thiotrophs (sulfide-oxidizing bacteria)**
  - **Cluster of closely related sequences**
  - **Present in all *Lophelia* colonies (n=6)**
  - **Dominant in two libraries from VK906/862**
  - **Related to sequences from shallow-water coral (89%)**
  - **Related to seep clam symbionts (86%)**
  - **Similar cluster found in Norwegian *Lophelia* (95–98% similar)**

Lophelia – 16S Clone Libraries

- **One clone is an unknown *Spirochete* (<90% similar to anything in the database)**

- **One clone was 99% similar to a cyanobacterium**




Alphaproteobacteria
Gammaproteobacteria
Tenericutes
Bacteroidetes
Taxon
0
10
20
30
40
50
60
70
80
90
100
VK906/862
VK826

Lophelia – 16S Clone Libraries

- **Differences between sites:**
  - **VK906/862**
    - **Proteobacteria, psychrophiles, thiotrophs**
    - **325m, temp >10 degrees C, delicate morph, no seep**
  - **VK826**
    - ***Tenericutes* (mycoplasmas)**
    - **500m, temp <10 degrees C, calcified morph, localized seep**

Summary

- **Culture data**
  - **No published study on diversity of cultured bacteria from *Lophelia***
  - **Bacterial groups include both those commonly cultured from shallow-water tropical corals and psychrophiles.**
  - **Unusual isolates are being characterized in depth to define new type species.**

Summary

- **Clone library data**
  - **Largest number of *Lophelia*-associated clones analyzed to date: 508**
    - **Norwegian paper = 340 clones, Med paper = 12 clones**
  - **Many novel sequences, but similar to coral-assoc**
  - ***Lophelia*-specific bacteria (Candidatus *Mycoplasma coralicola* and thiotroph cluster)**

Summary

- **Clone library data**
  - **Evidence of heterotrophic cyanobacteria (nitrogen fixers?)**
  - ***Lophelia*-associated bacterial communities are somewhat conserved but not as strictly as shallow-water corals**

Future Directions

Lophelia

- **Further characterization of cultured isolates**
  - **Employ variety of media to isolate more unusual bacteria**
- **Bacterial functional genes**
- **Vertical or horizontal transmission of symbionts?**
- **Other microbes**
  - **Archaea, fungi, viruses**

Future Directions

Deep-coral ecosystems

- Other deep-sea corals
- Coral-associated mobile fauna

DISCOVRE

http://fl.biology.usgs.gov/DISCOVRE/index.html

Diversity, Systematics, and Connectivity of Vulnerable Reef Ecosystems

U.S. Department of the Interior
U.S. Geological Survey

References

Friewald, A. Compiled from various sources. International Coral Reef Initiative. Cold water coral reefs: Global distribution of cold water coral reefs. http://www.icriforum.org/secretariat/cold/cold_water_map.jpe

Neulinger, S.C., J. Järnegren, M. Ludvigsen, K. Lochte, and W.-C. Dullo. 2008. Phenotype-specific bacterial communities in the cold-water coral Lophelia pertusa (Scleractinia) and their implications for the coral's nutrition, health, and distribution. Applied and Environmental Microbiology 74:7272–7285.

Penn, K, D. Wu, J.A. Eisen, and N. Ward. 2006. Characterization of bacterial communities associated with deep-sea corals on Gulf of Alaska seamounts. Applied and Environmental Microbiology 72(2):1680–1683.

Yakimov, M.M., S. Cappello, E. Crisafi, A. Trusi, A. Savini, C. Corselli, S. Scarfi, and L. Giuliano. 2006. Phylogenetic survey of metabolically active microbial communities associated with the deep-sea coral Lophelia pertusa from the Apulian plateau, Central Mediterranean Sea. Deep Sea Research 53:62–75.