Title
Back to the Sea: Seagrass As a Model System for Plant Microbiome Studies

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Back to the Sea: Seagrass As a Model System for Plant Microbiome Studies

Jonathan A. Eisen
UC Davis

January 22, 2018

Bilateral Joint Symposium
Academia Sinica & UC Davis

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Lessons Learned When You Work on Something You Know Nothing About

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Seagrass Microbiome Project
Lesson 1:

Good Colleagues Are A Good Thing

Sept. 2010: Moore Foundation MMI RFI

GBMF's Marine Microbiology Initiative (MMI) -- Request for Ideas (RFI)

Marine Microbiology Request for Ideas <mmi.rfi@moore.org> 9/21/10

to

The Gordon and Betty Moore Foundation's Marine Microbiology Initiative (MMI) has issued a Request for Ideas (RFI) for marine microbiology and marine microbial ecology research. We are looking to identify the most promising opportunities where a strategic, focused effort over the next five years will help to break open 'black boxes' in the field and take understanding of marine microbial communities to a new level.

More information about the RFI can be found here: http://www.moore.org/mmi-rfi.aspx. Researchers interested in contributing an RFI for consideration should submit a two-page idea summary by **November 8, 2010**.

Please forward this to anyone who might be interested. Thank you in advance for helping us spread the word, and our apologies for cross-postings.

– The Marine Microbiology Initiative team

Sept. 2010: Moore Foundation MMI RFI

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Marine Microbiology Request for Ideas <mmi.rfi@moore.org> 9/21/10

to

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understanding

More informat ed in
contributing a

2 Page Idea Summary

Due November 8, 2010

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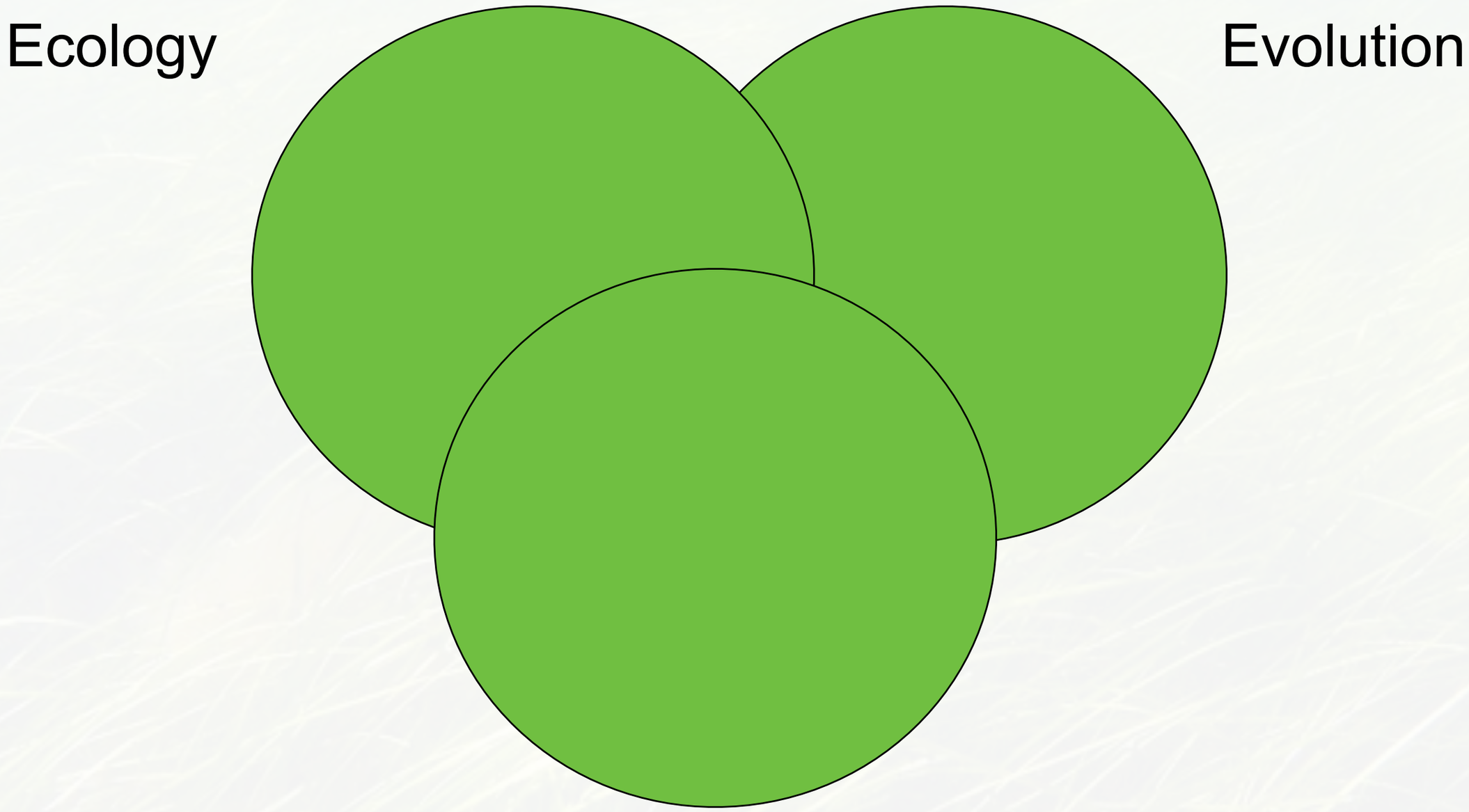
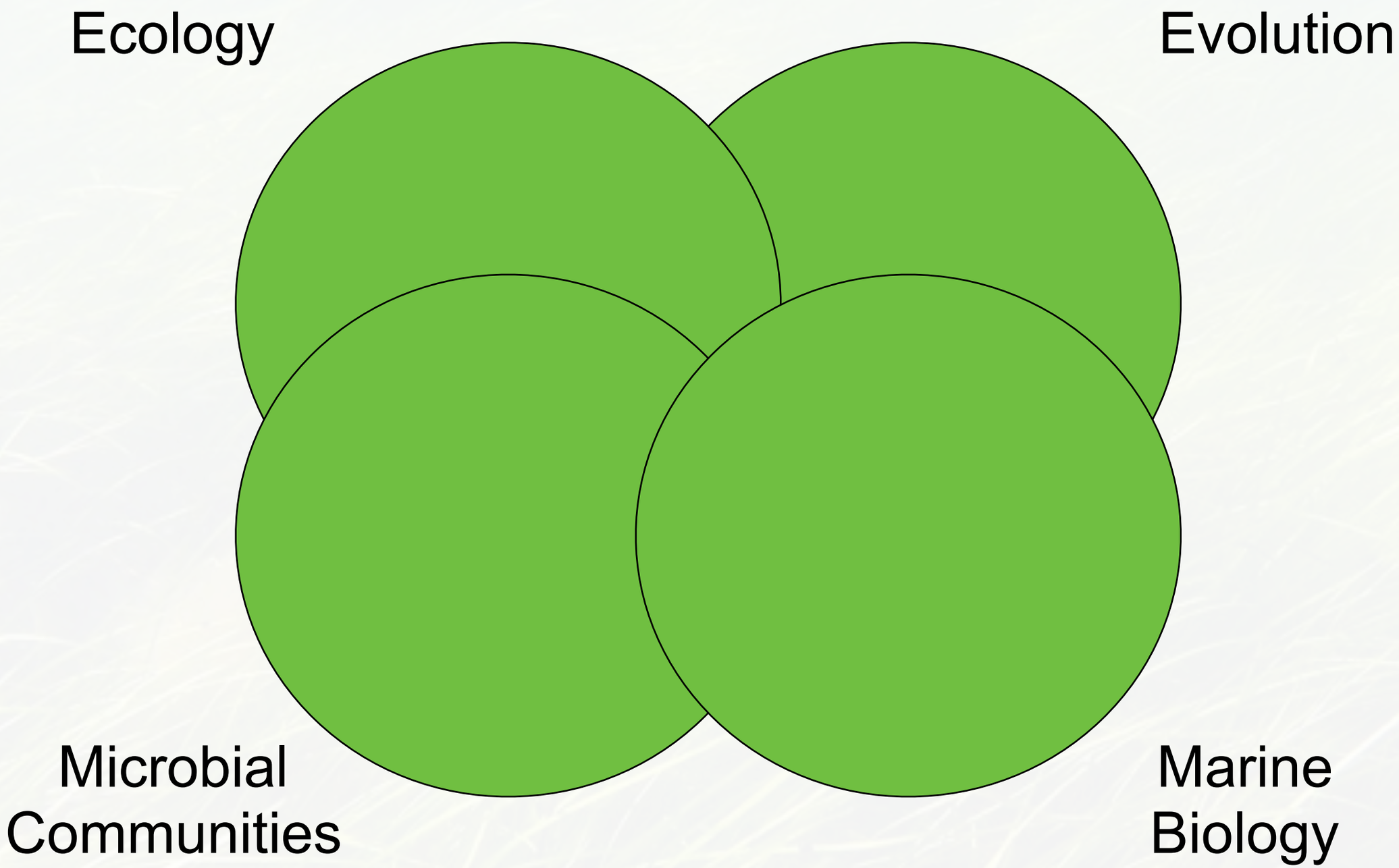
– The Marine Microbiology Initiative team

Eisen Lab Microbiome Topics



Eisen Lab Topics



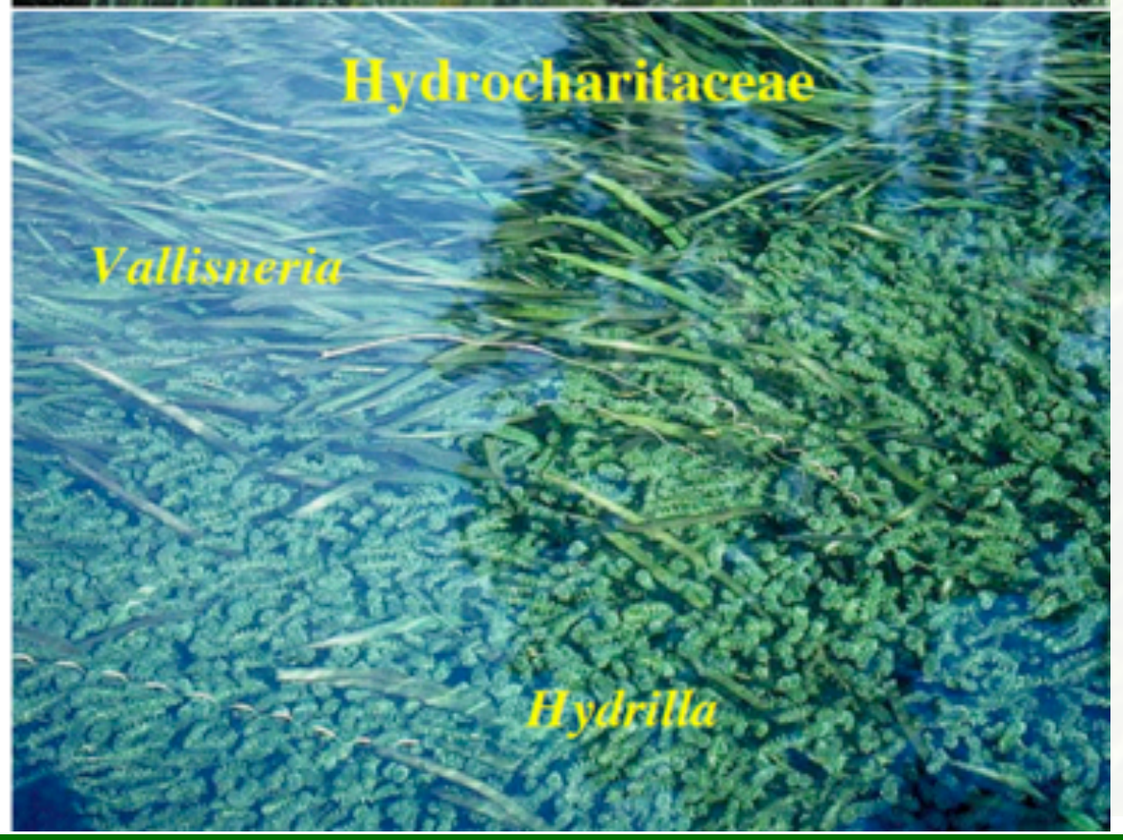
Contacted Colleagues

- Jessica Green
- Katie Pollard
- Jenna Lang (in my lab)

- Multiple calls, not sure what to propose

- Also - extremely busy teaching BIS2C w/ 4 lectures per week and 700 students

Oct. 2010 Jim Doyle: Aquatic Monocots



Oct. 2010 Jim Doyle: Aquatic Monocots

Why monocots lose 2° growth.

- probably an adaptation to aquatic habitat
- no need for wood for conductor of support when you live in H_2O
- supported by fact that basal monocots are aquatic

Araceae

- some ever went into ocean

Palms - tree like but not secondary growth

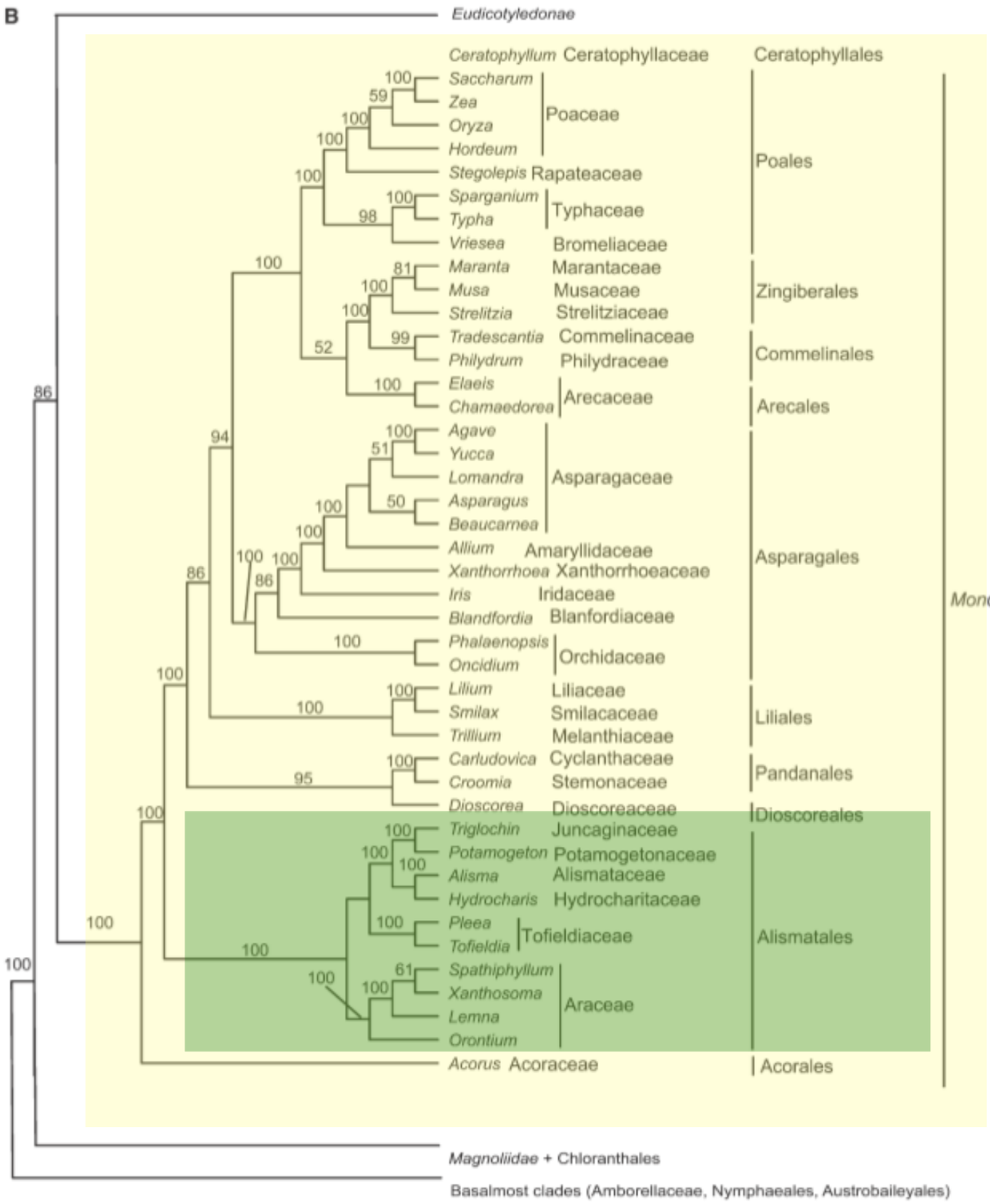
Oct. 2010 Jim Doyle: Aquatic Monocots

Alismatales: "sea grasses": marine monocots

Zosteraceae

Zostera **(eelgrass)**

Seagrass w/in Monocots

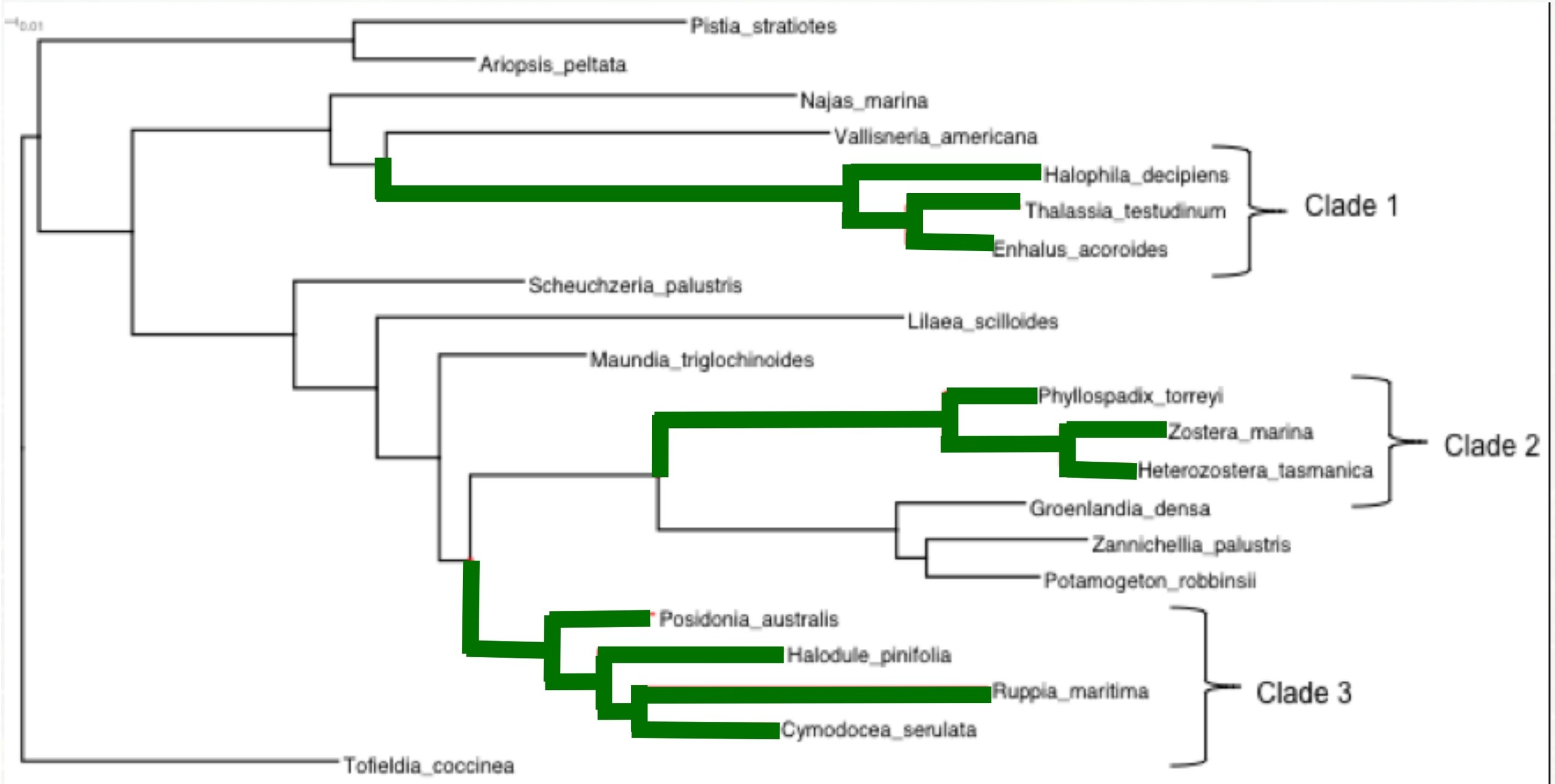


Dicots

Monocots

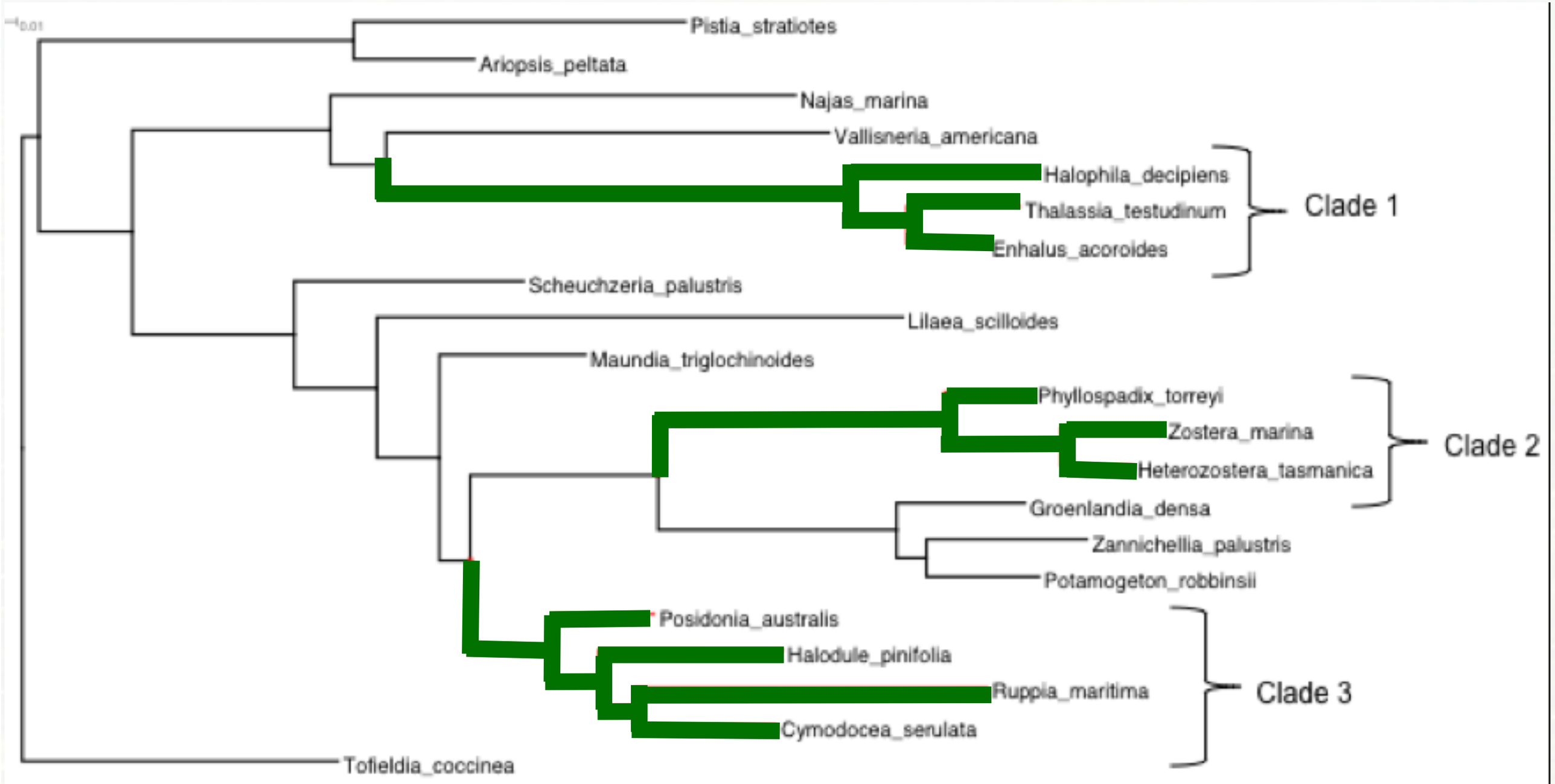
Alismatales

Seagrasses w/in Alismatales



Tree inferred by Jenna Lang based from rbcL sequences using RaxML

Seagrasses Polyphyletic



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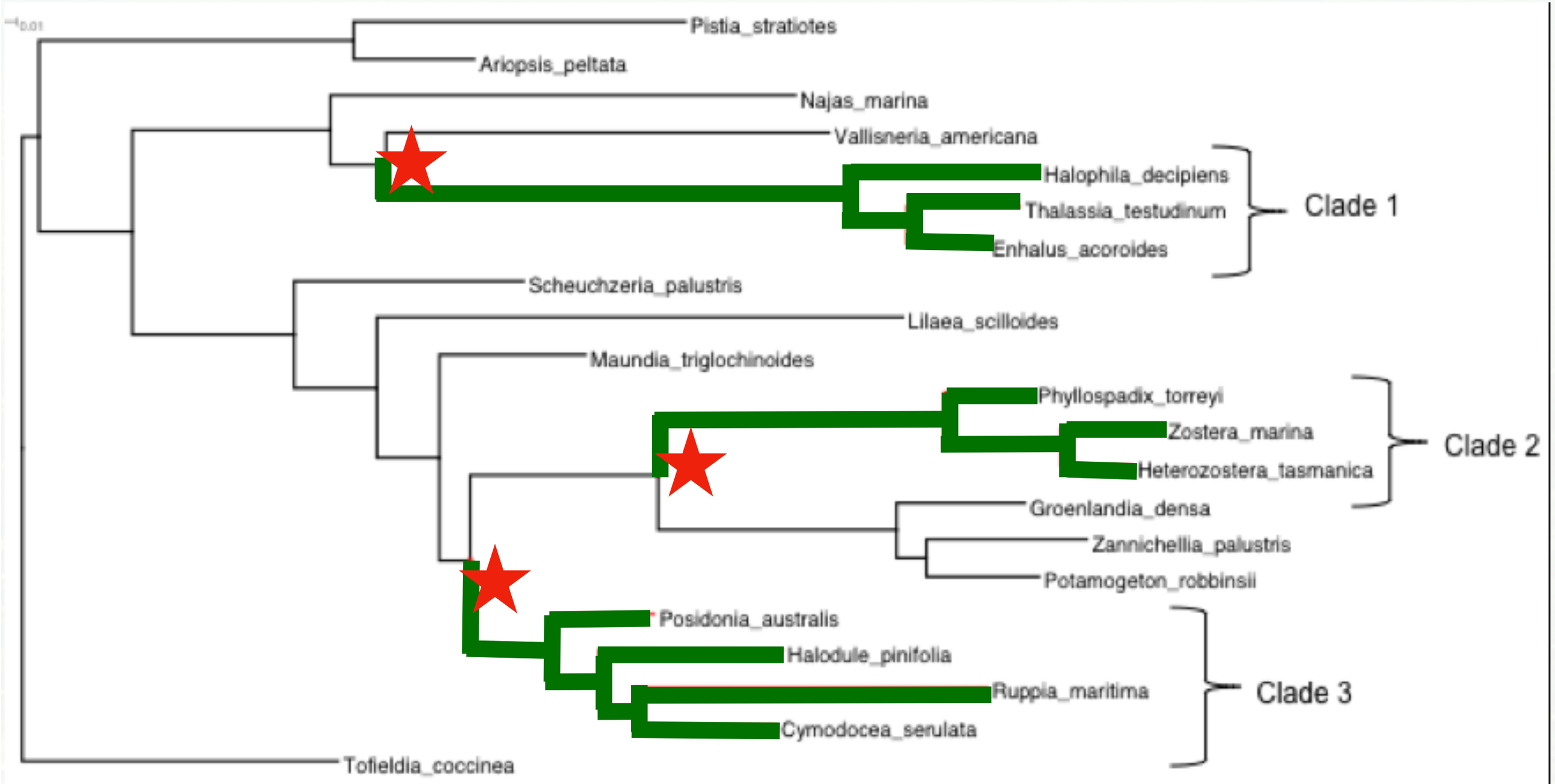
Seagrasses: 3 Invasions of Marine



Tree inferred by Jenna Lang based from rbcL sequences using RaxML

Seagrass Diversity

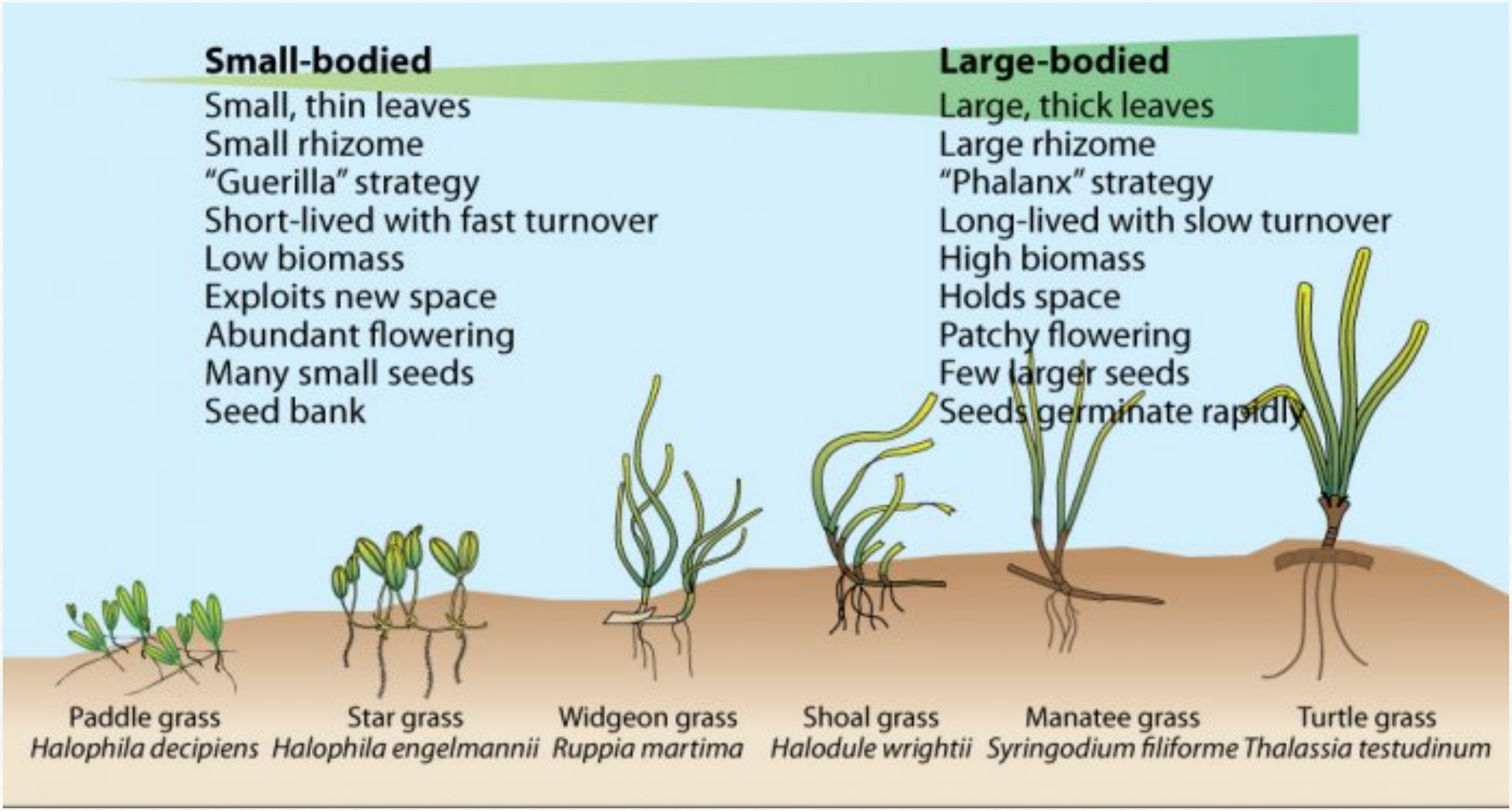
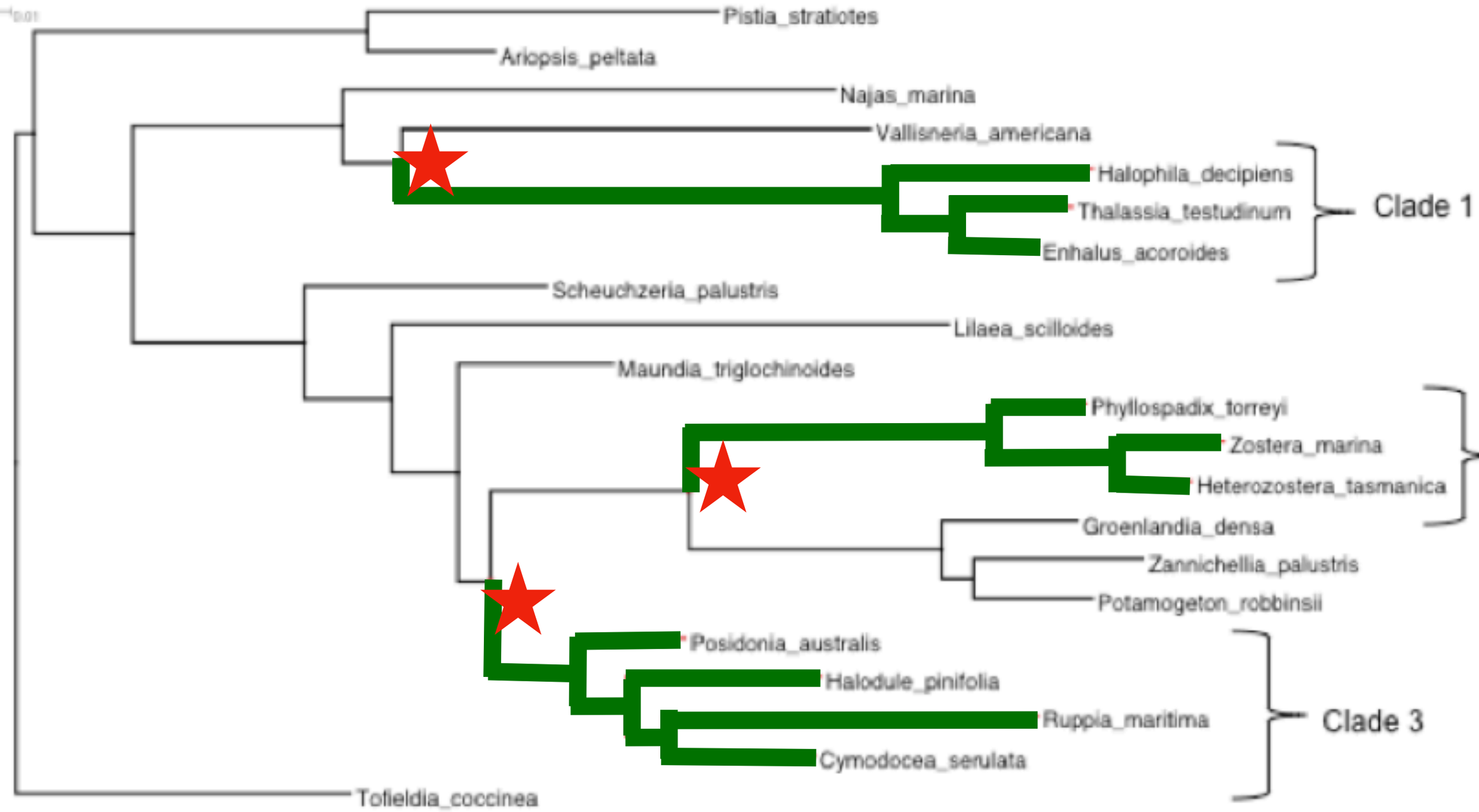


Image from Reynolds PL. Seagrass and Seagrass Beds
http://ocean.si.edu/seagrass-and-seagrass-beds

Seagrasses: Significant Convergence



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Seagrass Microbiomes?

- **Many reasons for interest**
  - **Convergence of microbiomes?**
  - **Comparison to other monocots**
  - **Adaptations to salt / marine environment**
- **But …**
  - **No experience in our mega-group working with seagrass …**
  - **Little literature on seagrass microbiomes**
- **So? ….**

Seagrass Microbiome Project
Lesson 1:

Good Colleagues Are A Good Thing

Jay Stachowicz - Seagrass EcoEvo

THE STACHOWICZ LAB

Home Research People Publications Outreach Lab Alumni

Marine Community Ecology

Letter to Jay Stachowicz

Jay - apologies in advance for a long email ...

I am writing to see if you would have any time in the next week to discuss a somewhat last minute idea I am working on for a two page white paper I want to send to the Moore Foundation.

They have out a call for short idea papers (http://www.moore.org/mmi-rfi.aspx) for new research areas in marine microbiology and if they like your white paper might ask for a full proposal. The idea I and a collaborator (actually someone I think you may know - Jessica Green) have is to propose studies of the diversity and function of microbes living in and on marine plants/algae. I think this could be of scientific value and interest both from a comparative point of view as well as in terms of the marine organisms in question. There is some literature on this, as you may be aware, but not an enormous amount. What I have found though suggests that there may be some important and interesting functions yet to be characterized associated with the microbiota living in association with these organisms.

Alas, I know little about the host organisms here - though I have done work on microbes living in and on lots of other species. I know that you have done some work on eelgrass and possibly other marine plants/algae and may even have done some work on microbes with which they are associated (I think a student mentioned this at some point, but not sure anymore what they said).

Anyway - I was wondering if you had any time to talk briefly about this idea in the next week and whether you would be interested in participating in the white paper.

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Jay Stachowicz - Seagrass Guru

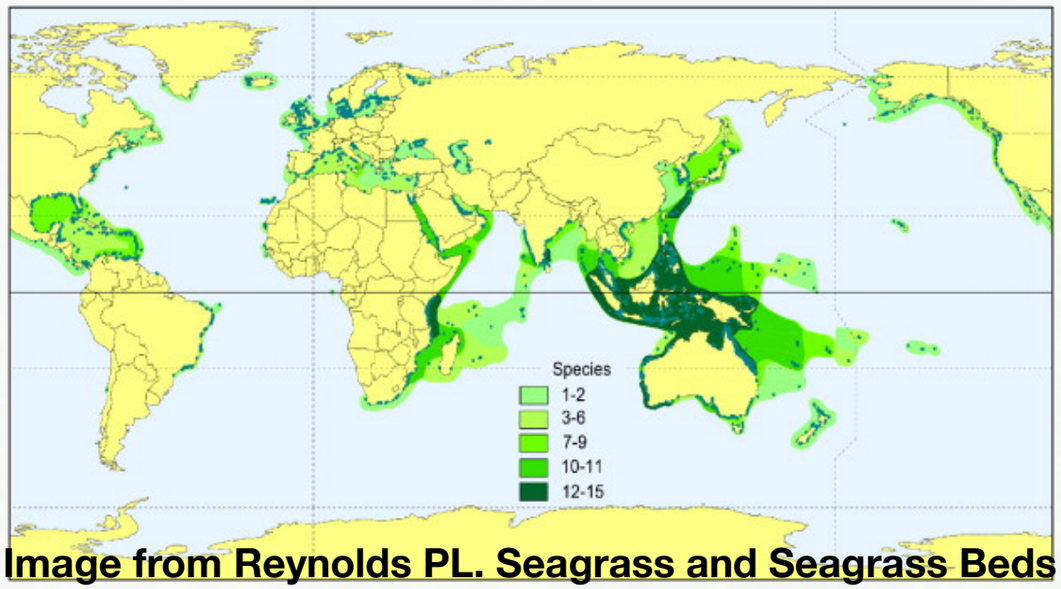


Image from Reynolds PL. Seagrass and Seagrass Beds
http://ocean.si.edu/seagrass-and-seagrass-beds

- Seagrass Importance
- Ecosystem Structure
- Living Habitat
- Foundation of Food Webs

*Zostera marina (*eelgrass*)* is the most abundant seagrass throughout the northern hemisphere

Slide from Jay Stachowicz

Eelgrass provides shelter (and food) for many animals

Slide from Jay Stachowicz

The Seagrass Microbiome Project

- **Aim 1**: How have the microbial communities associated with seagrasses co- evolved with their hosts and what roles in the past and currently do microbes play in adaptations of plants to fresh and marine water life?

- **Aim 2**: What drives the community assembly of the seagrass microbiome, and specifically within the *Zostera marina* model system?

- **Aim 3**: What role does the microbial community play in the functional ecology of the *Zostera marina* (with a specific focus on sulfur and nitrogen metabolism and primary production)?


Jonathan Eisen


Jay Stachowicz


Jessica Green


Jenna Lang

GORDON AND BETTY MOORE FOUNDATION

The Seagrass Microbiome Project

- **Aim 1**: How have the microbial communities associated with seagrasses hosts and what roles in the past and cu ay in adaptations of plants to fresh and marine water life?

Evolution

- **Aim 2**: W munity assembly of the seagrass mi cifically within the *Zostera marina* model system?

Ecology

- **Aim 3**: W crobial community play in the functional ec *marina* (with a specific focus on sulfur an m and primary production)?

Function


Jonathan Eisen


Jay Stachowicz


Jessica Green


Jenna Lang

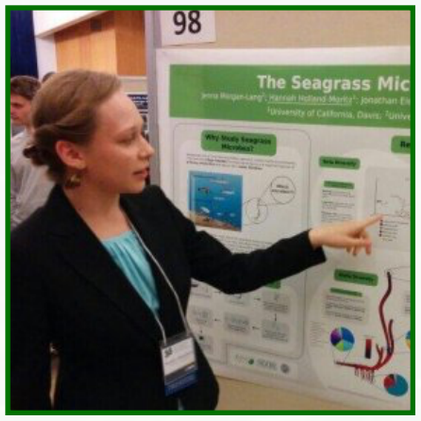
GORDON AND BETTY MOORE FOUNDATION

Seagrass Microbiome Project
Lesson 2:

Location Matters
Except When it Doesn't

Where To Start When You Know Nothing?

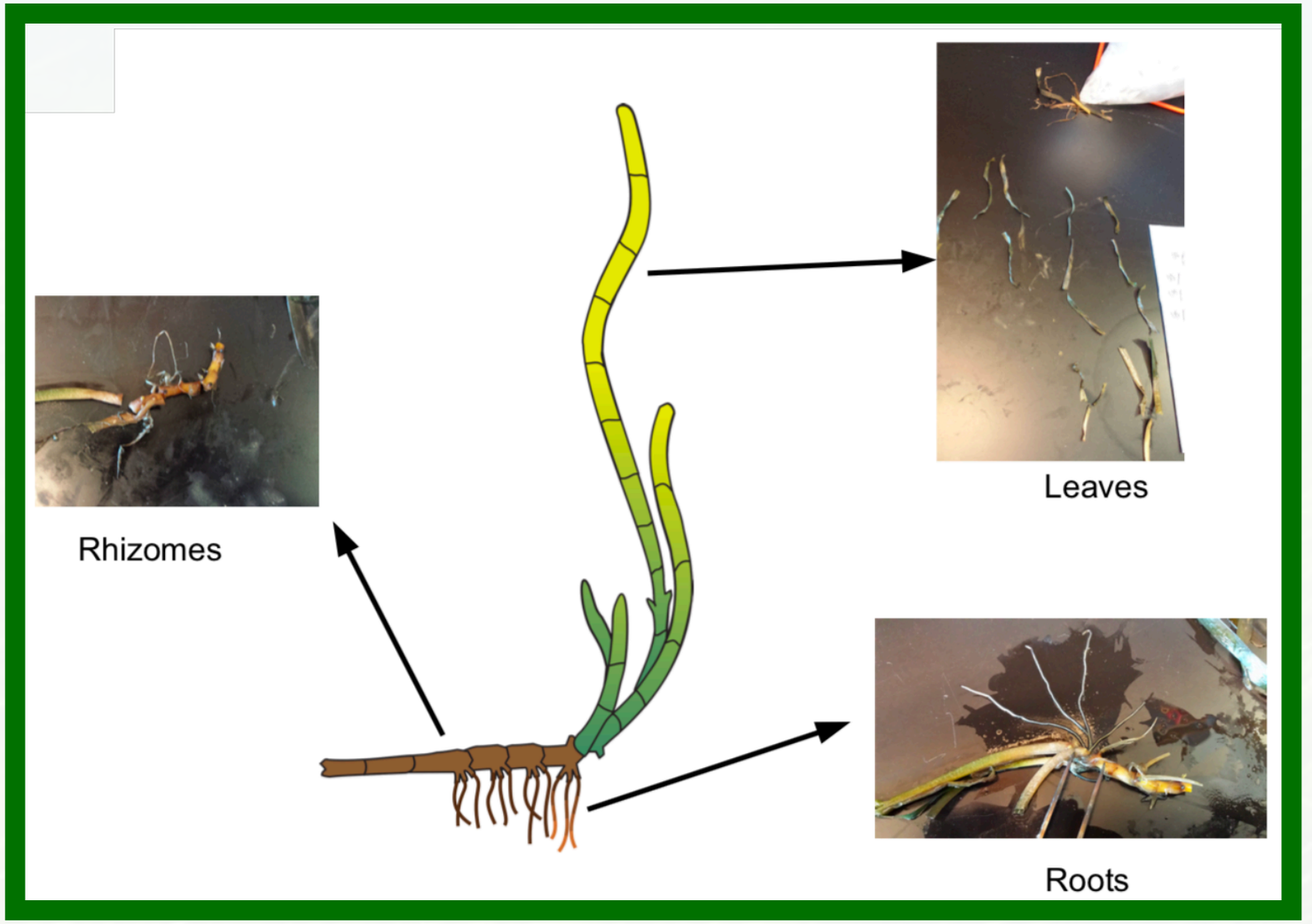
Intraplant Microbiome Biogeography



Hannah Holland-Moritz

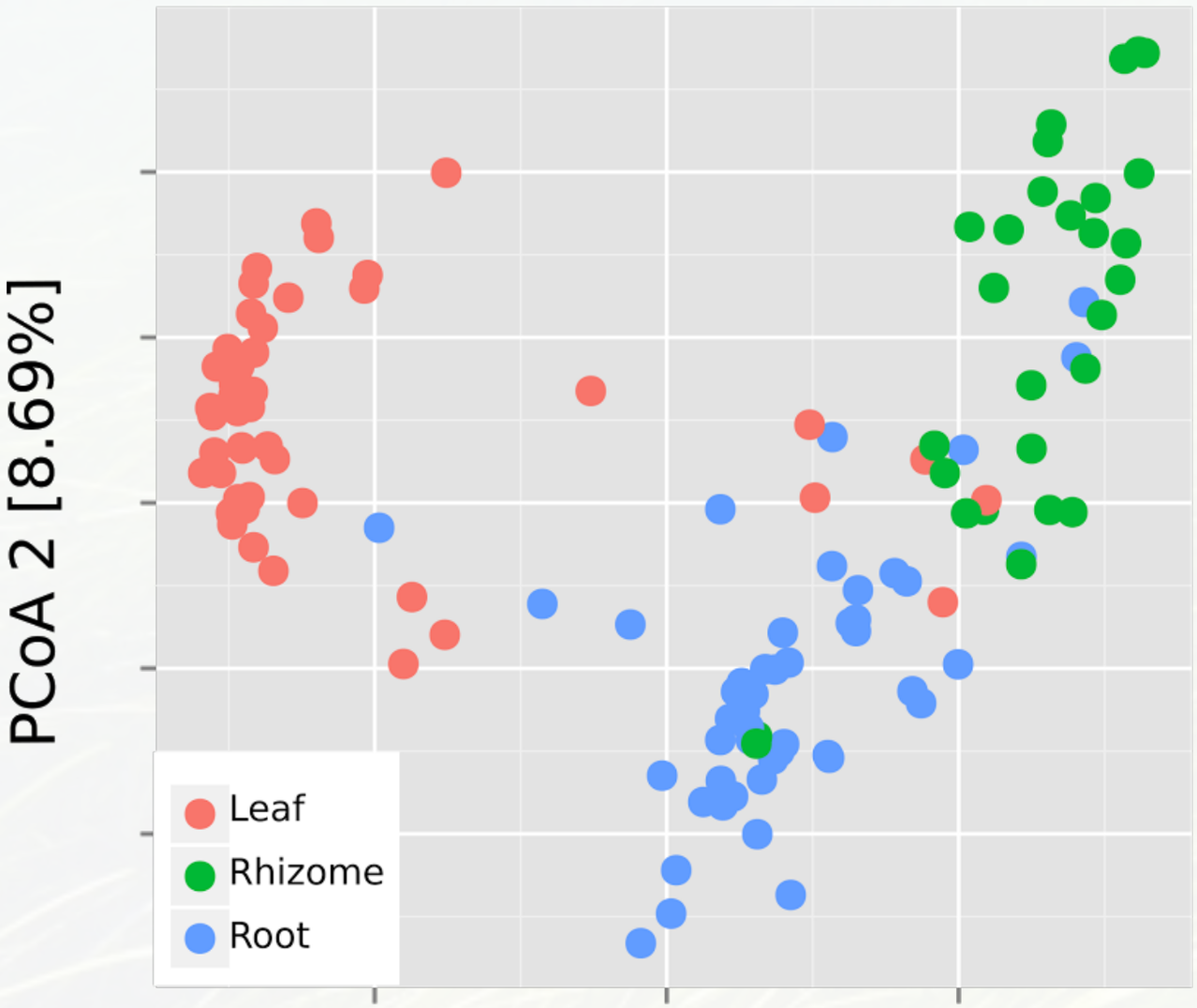
Ruth Lee

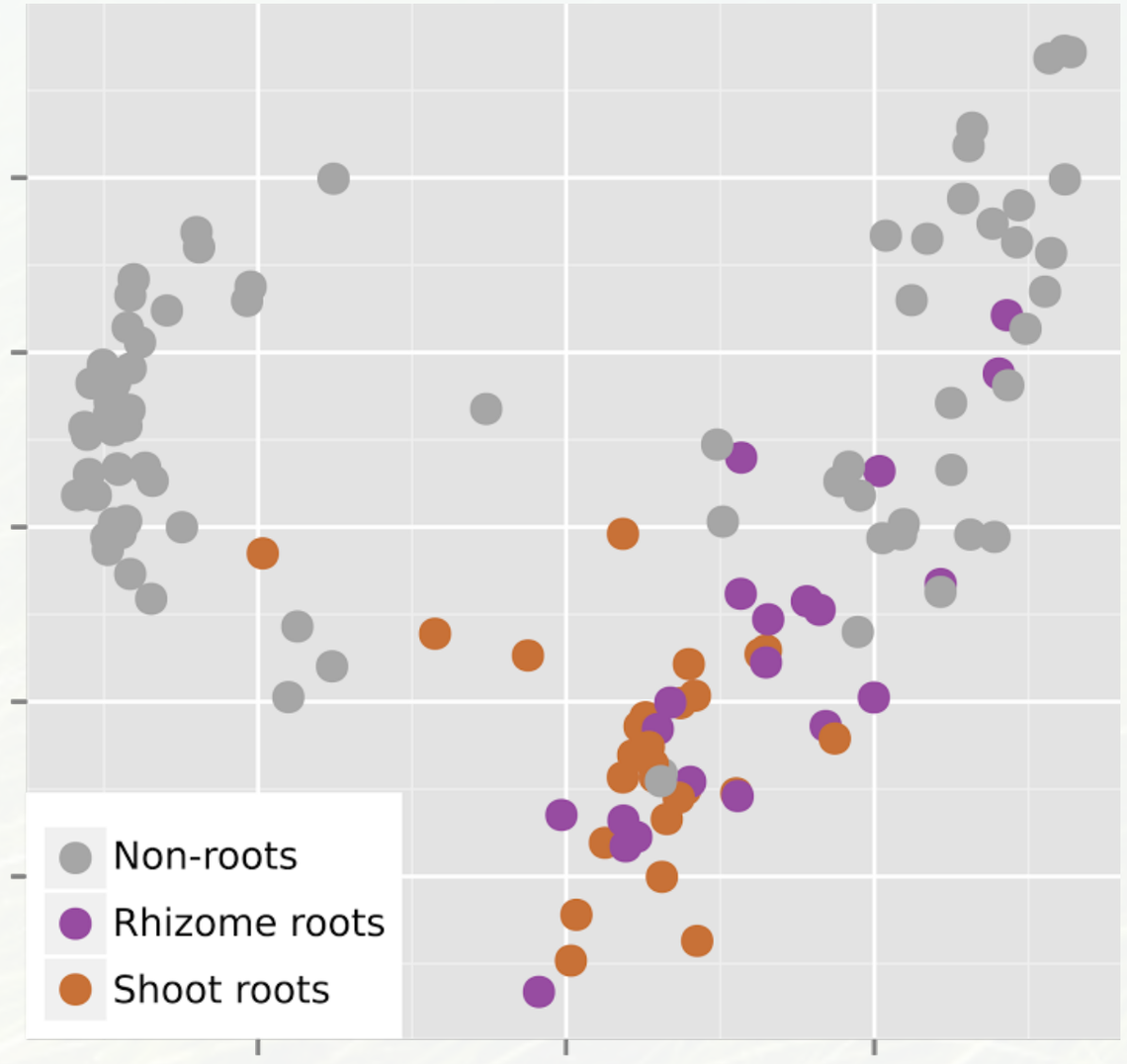
Jenna Lang



rRNA gene PCR, sequencing, informatics

Rhizome Roots vs. Shoot Roots vs. Leaf





PCoA 1 [26.88%]

Variation in microbial community composition in *Z. marina*. PCoA plot of weighted Unifrac distances between samples. Communities cluster by tissue type (PERMANOVA, $p < 0.001$). Within root samples, rhizome roots differ from shoot roots (PERMANOVA, $p < 0.001$).

Location Matters Except When it Doesn't

- There is more distinct micro-variation below-ground than above
- Proximity to the shoot is an important indicator of community composition (and possibly community function)
- Predicted sulfur cycling microbes dominate all communities regardless of location

The Seagrass Microbiome Project

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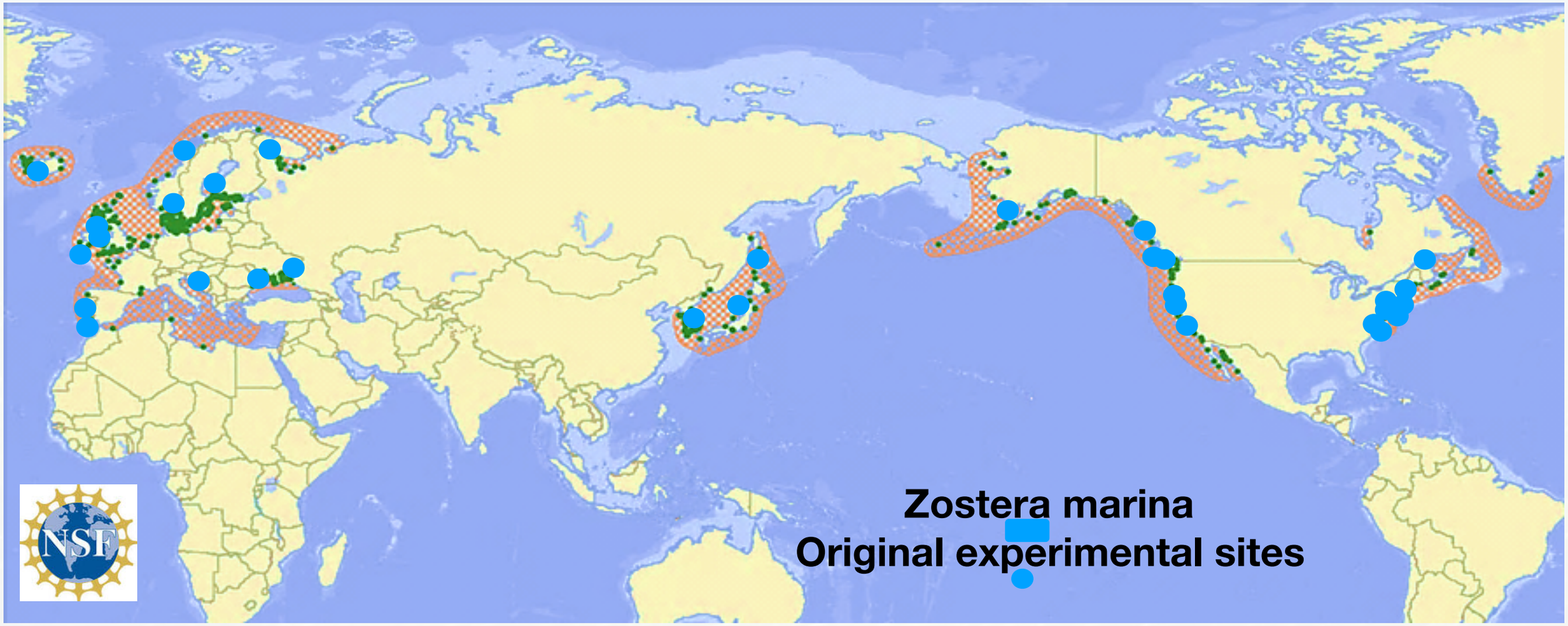
Ecology

- **Aim 3**: What role does the microbial community play in the functional ecology of the *Zostera marina* (with a specific focus on sulfur and nitrogen metabolism and primary production)?

Zostera Experimental Network (ZEN)

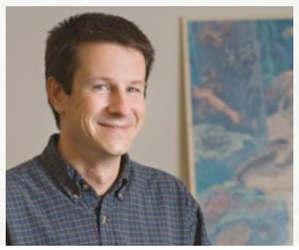


- 40 Sites in 24 countries
- Eelgrass genetic composition
- Eelgrass above and below ground biomass
- Associated epifauna and infauna

http://zenscience.org

ZEN
Zostera Experimental Network

Emmett Duffy

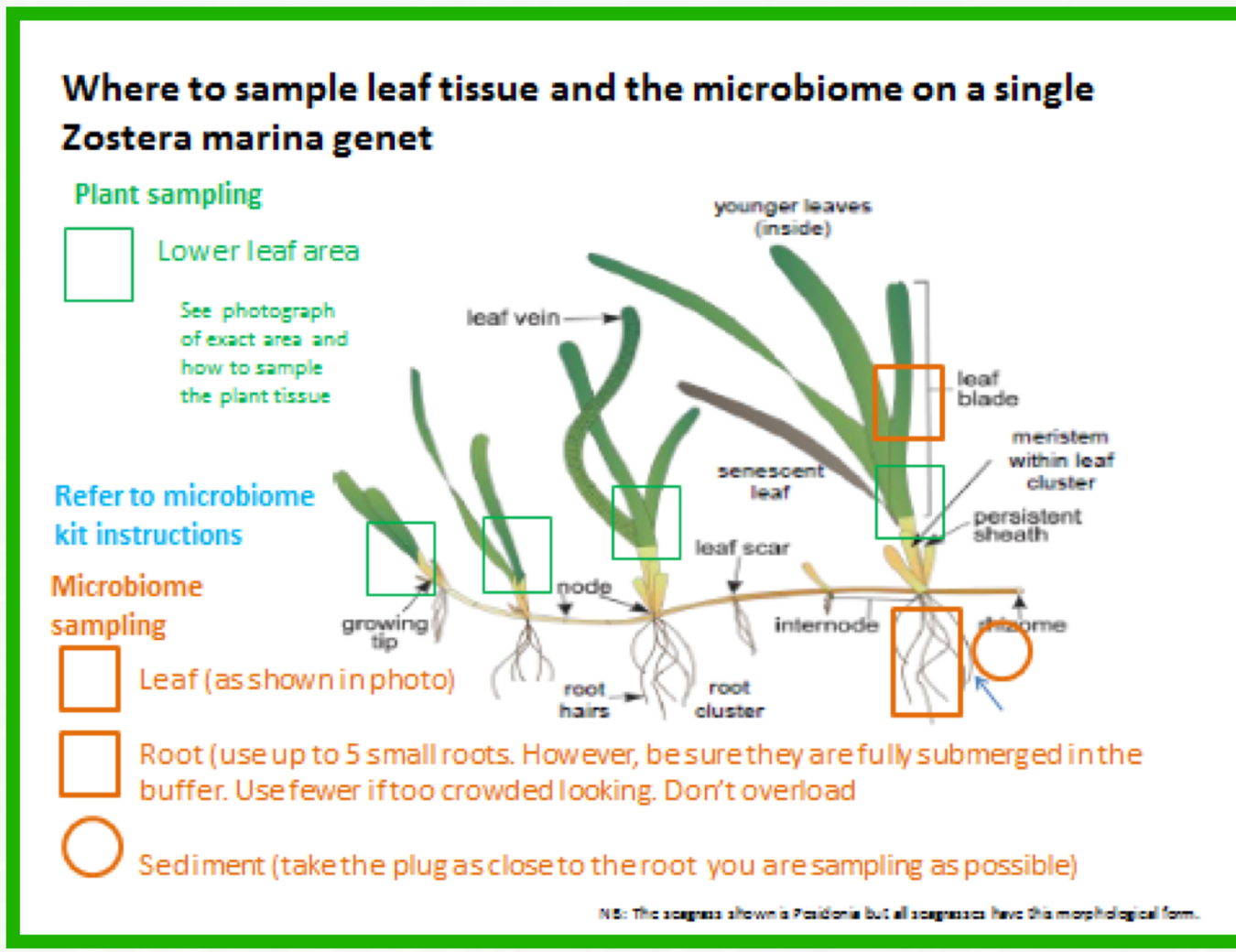


Jay Stachowicz

Pamela Reynolds

Kevin Hovel

Seagrass Microbiome ZEN Kit

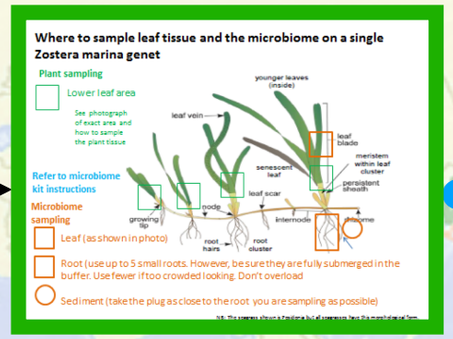


$25
custom filters
3D-printed stand

Jenna Lang

Russell Neches

ZEN Microbiome Sampling

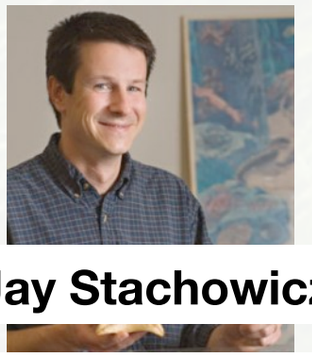


- Sent kits
- Asked to sample leaves, roots, sediment and water

http://zenscience.org

ZEN
Zostera Experimental Network

Emmett Duffy



Jay Stachowicz

Pamela Reynolds

Kevin Hovel

Seagrass Microbiome Project
Lesson 3:

Good Communities are a Good Thing

ZEN Microbiome Sampling

DNA Extraction

rRNA Gene PCR and sequencing

Informatics

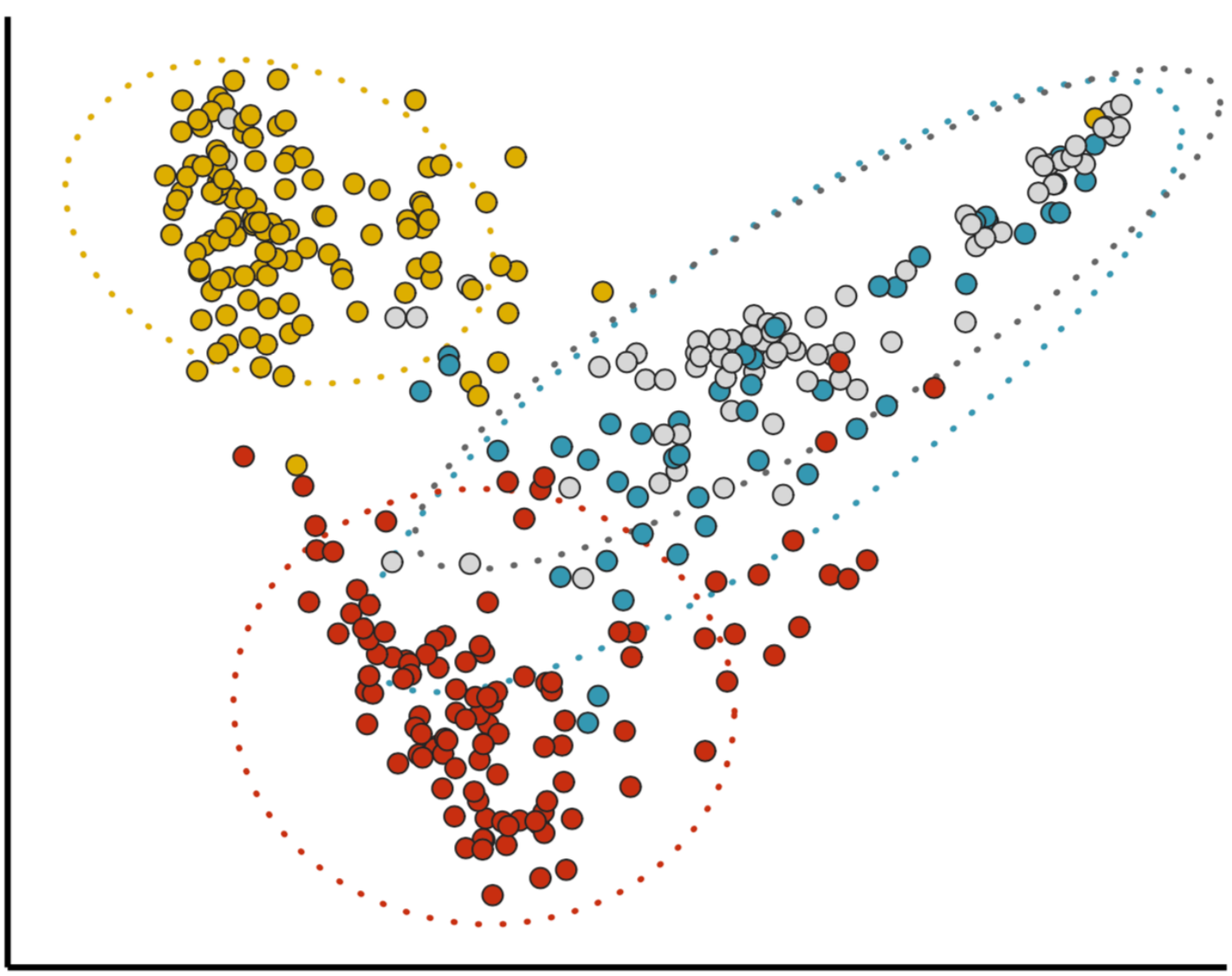
Fahimipour AK, Kardish MR, Lang JM, Green JL, Eisen JA, Stachowicz JJ. 2017. Global-scale structure of the eelgrass microbiome. Appl Environ Microbiol 83:e03391-16. https://doi.org/10.1128/AEM.03391-16.

Ashkaan Fahimipour

Melissa Kardish

Jenna Lang

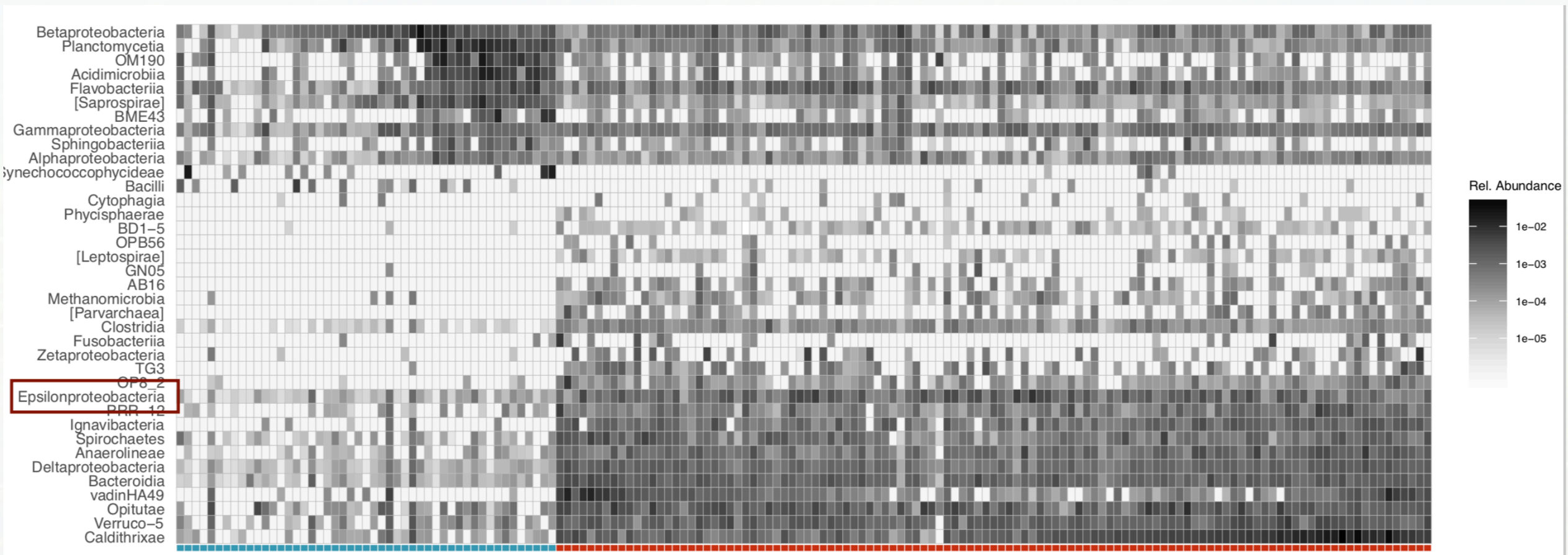
Global Structure of Eelgrass Microbiome



- Leaf, roots and sediment different
- Leaves resemble water
- Leaves more similar to local water

Fahimipour AK, Kardish MR, Lang JM, Green JL, Eisen JA, Stachowicz JJ. 2017. Global-scale structure of the eelgrass microbiome. Appl Environ Microbiol 83:e03391-16. https://doi.org/10.1128/AEM.03391-16.

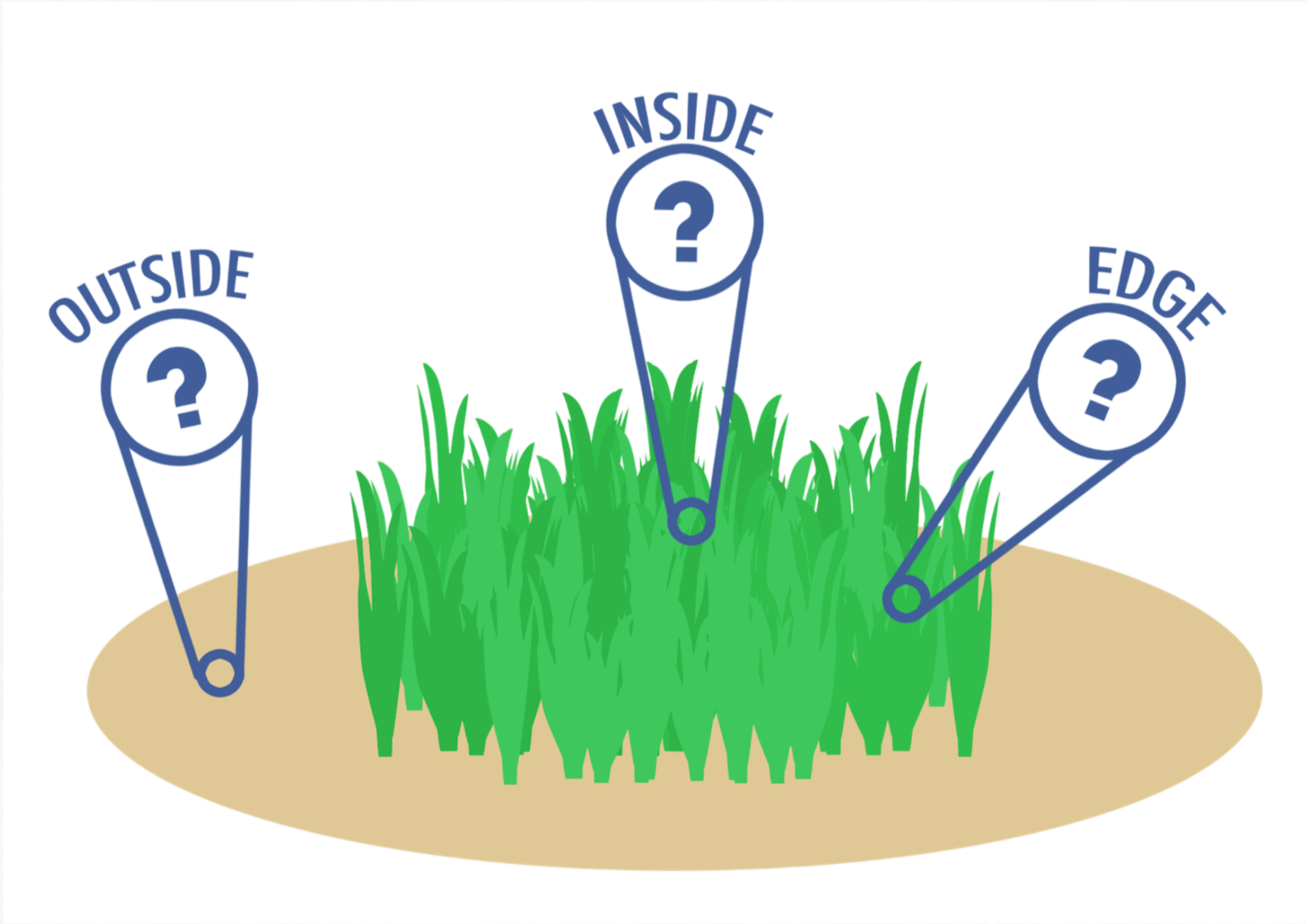
Sulfur Metabolism Enriched on Roots



Sulfurimonas – sulfur oxidizers

Fahimipour AK, Kardish MR, Lang JM, Green JL, Eisen JA, Stachowicz JJ. 2017. Global-scale structure of the eelgrass microbiome. Appl Environ Microbiol 83:e03391-16. https://doi.org/10.1128/AEM.03391-16.

Edge Effects: Does in Matter Where Plants Are?



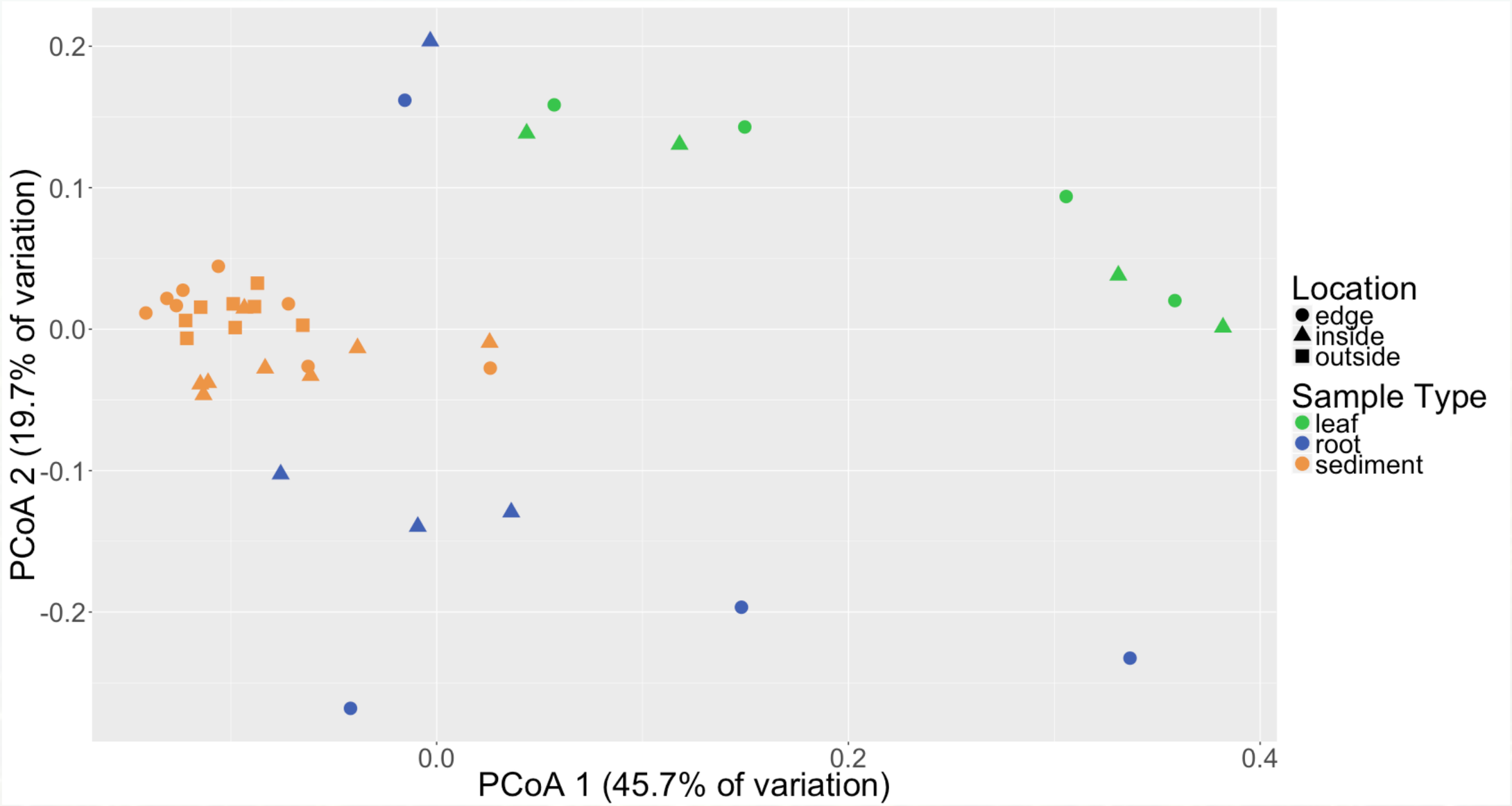
Ettinger CL, Voerman SE, Lang JM, Stachowicz JJ, Eisen JA. (2017) Microbial communities in sediment from Zostera marina patches, but not the Z. marina leaf or root microbiomes, vary in relation to distance from patch edge. PeerJ 5:e3246 https://doi.org/10.7717/peerj.3246


Cassie Ettinger


Sofie Voerman


Jenna Lang

Edge Effect in Sediment but Not Plant Microbiomes

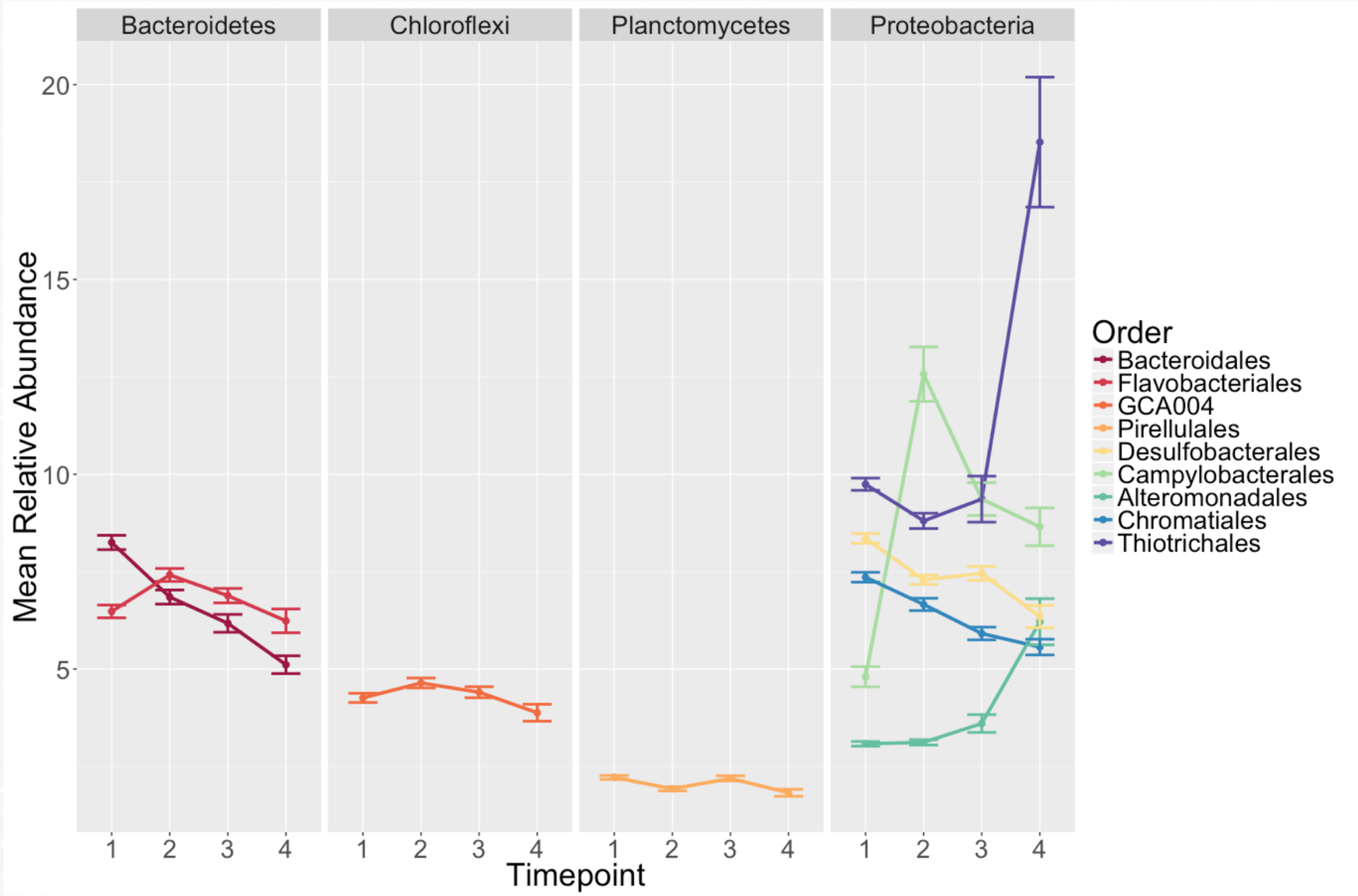


- Plant parts (root, leaf) and near-by sediment different from each other.
- Edge effects not seen for plant microbiomes
- Edge effect seen for sediment

Seagrass Microbiome Project
Lesson 4:

Disturbance Can Be Good and Bad

Succession During Ammonification



Changes appear driven by sulfur cycling w/ decreases in sulfur reducers (Desulfobacterales) and corresponding increases in sulfide oxidizers (Alteromonadales and Thiotrichales).

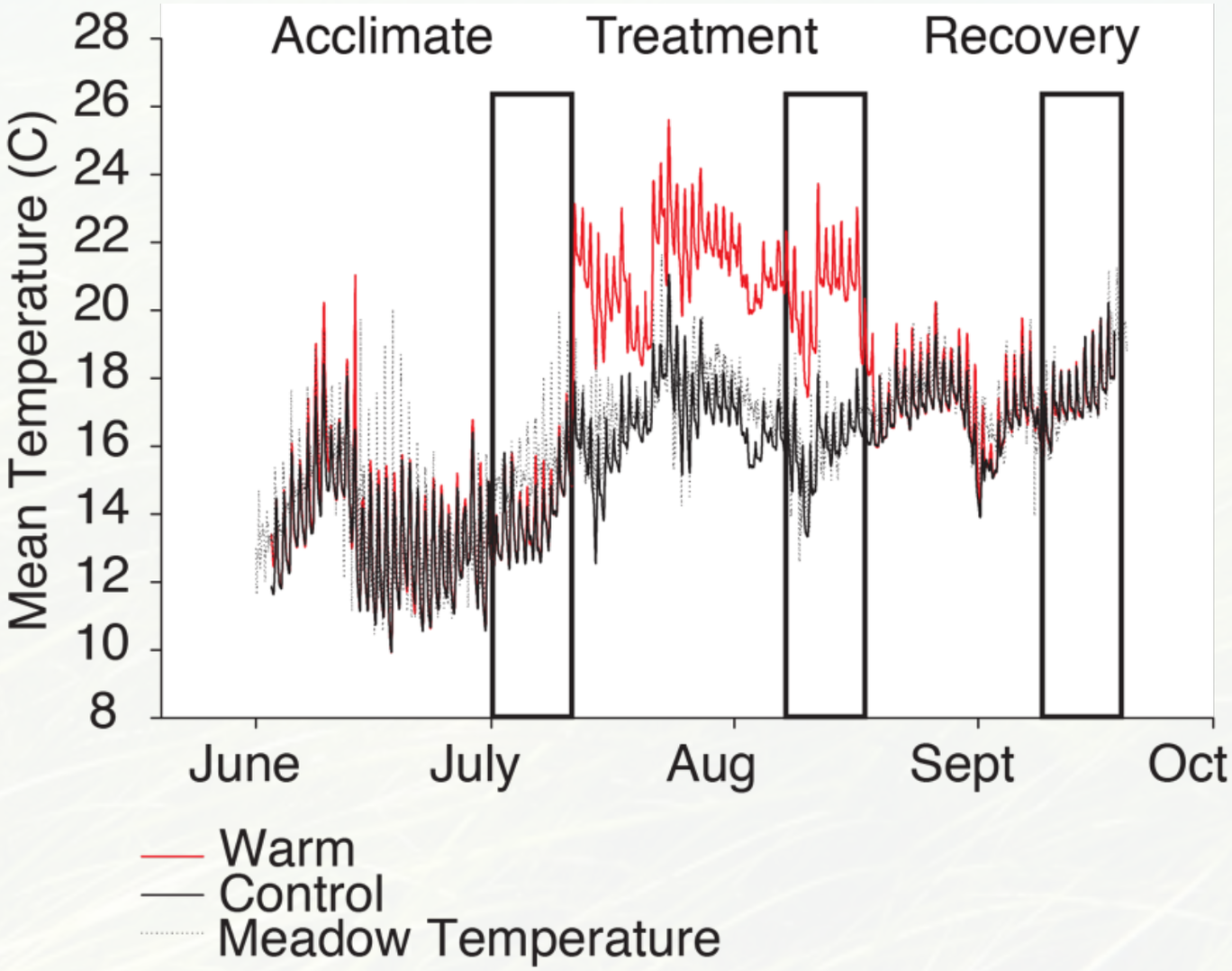
Ettinger CL, Williams SL, Abbott JM, Stachowicz JJ, Eisen JA. (2017) Microbiome succession during ammonification in eelgrass bed sediments. PeerJ 5:e3674 https://doi.org/10.7717/peerj.3674


Cassie Ettinger


Jessica Abbott


Susan Williams

Moderate 4.5° C increase



Alana Firl

Laura Reynolds

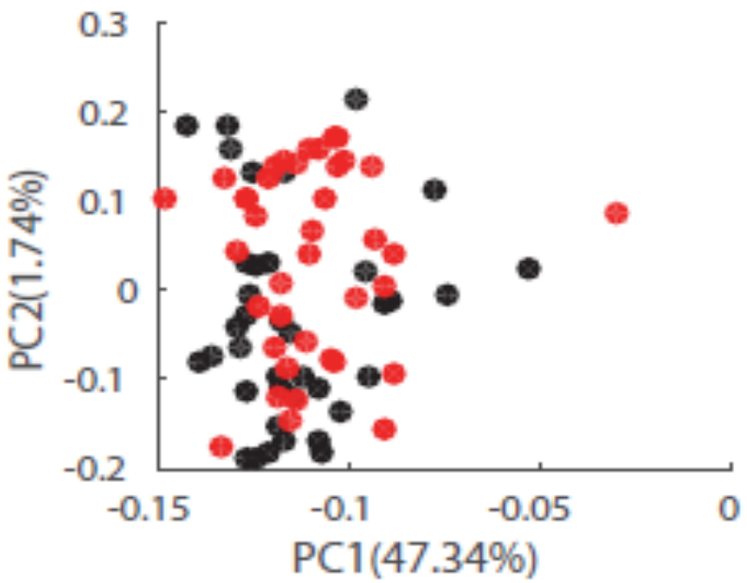
Katie DuBois

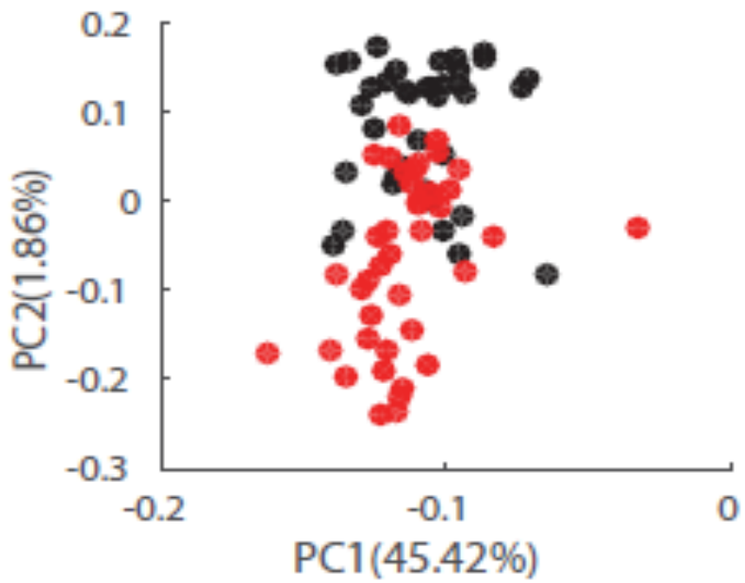
Jessica Abbott

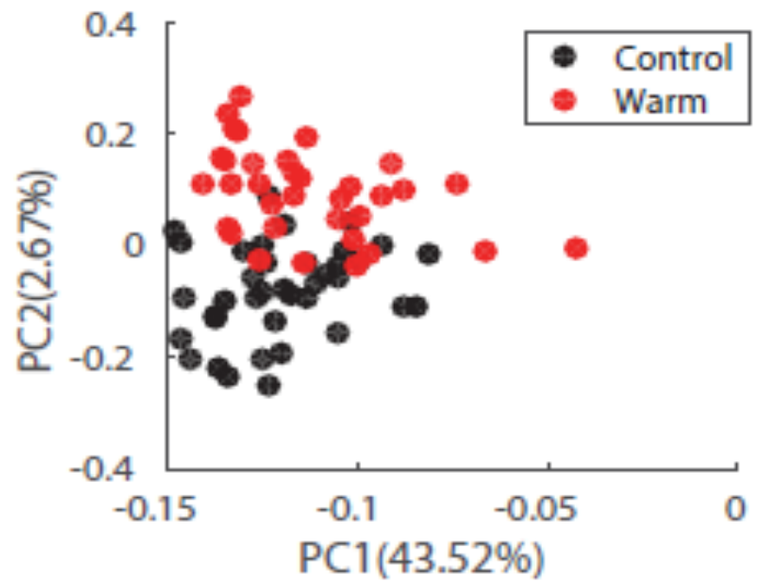
Susan Williams

Leaf microbiomes exhibit T°-related shifts

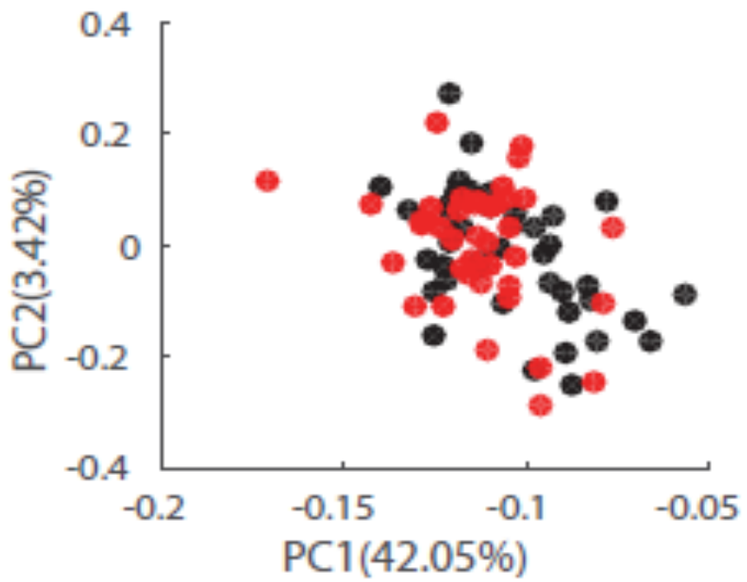
Leaves

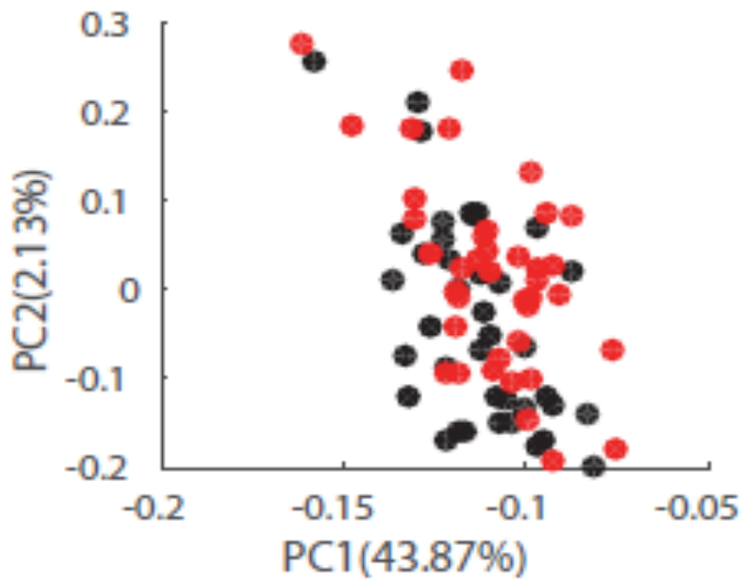


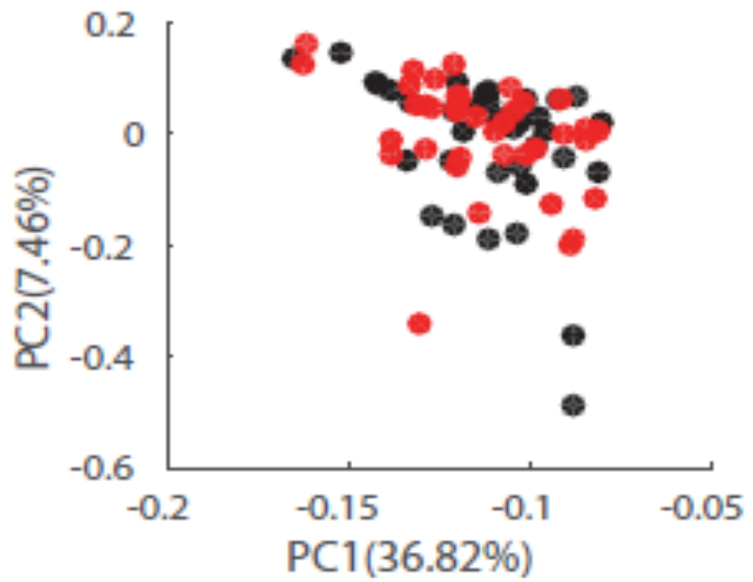




Roots







Seagrass Microbiome Project
Lesson 5:

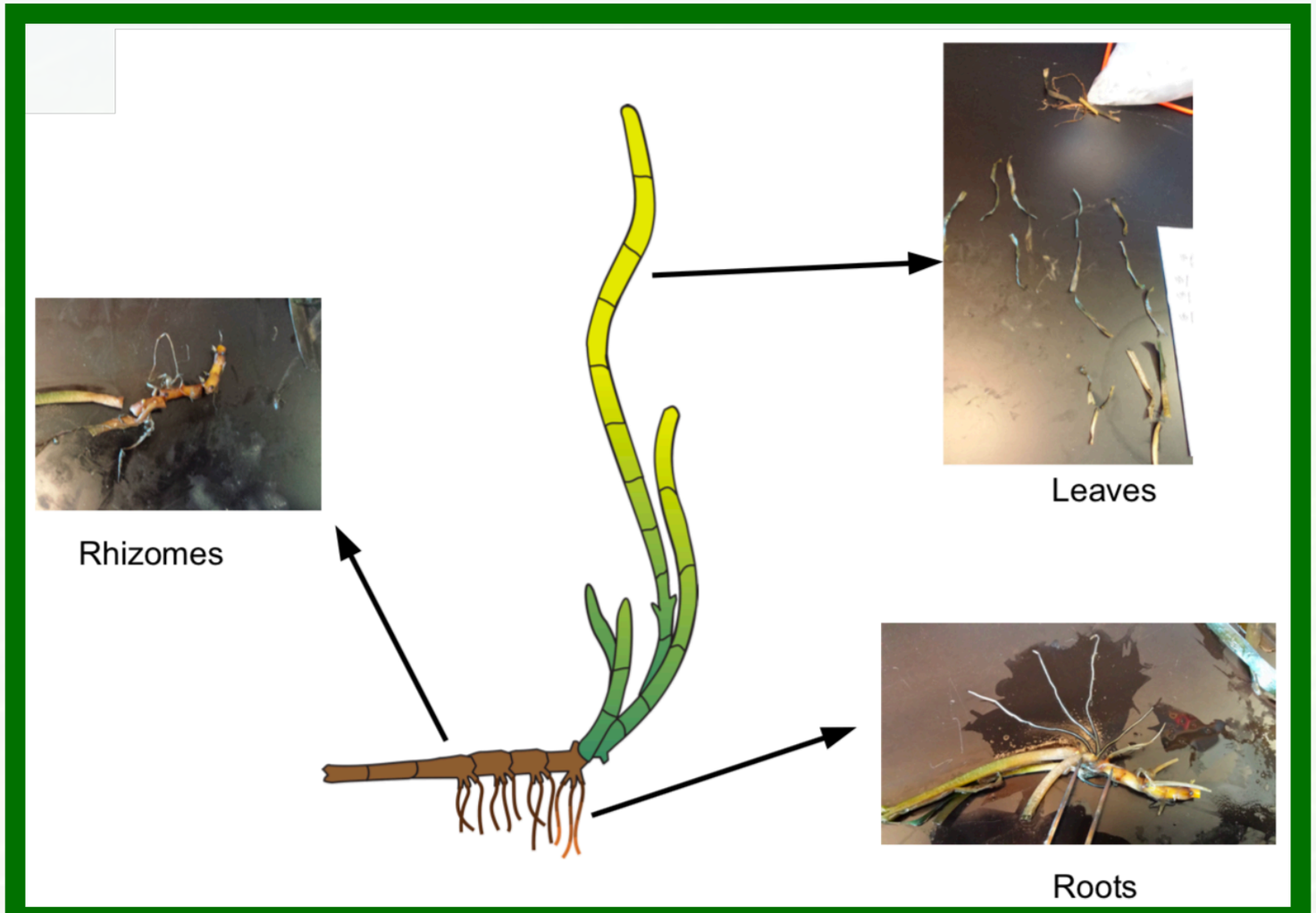
Don’t Forget Your Roots

Intraplant Sampling Metagenomics

Laura Vann

Guillaume Jospin

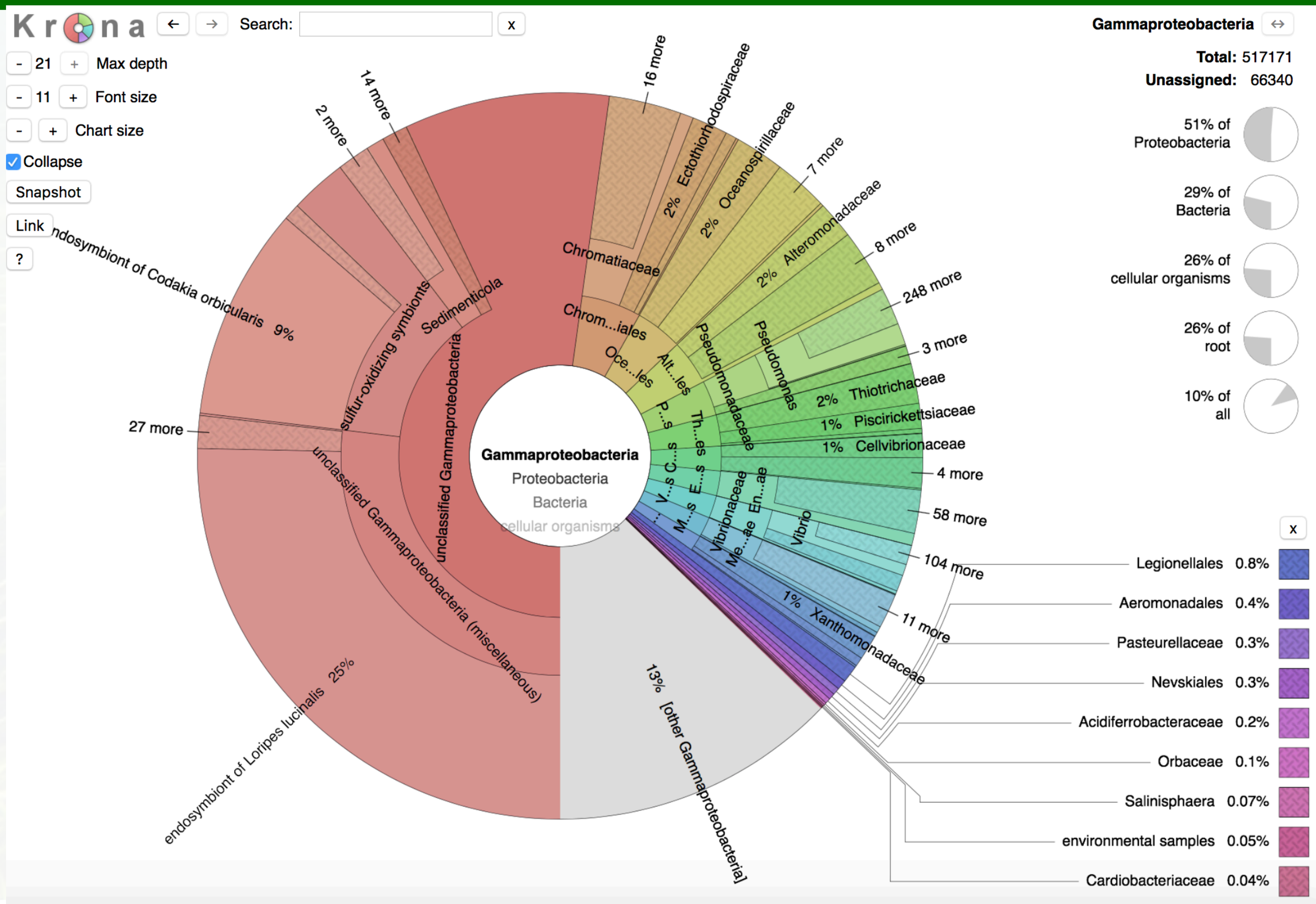
Melissa Kardish



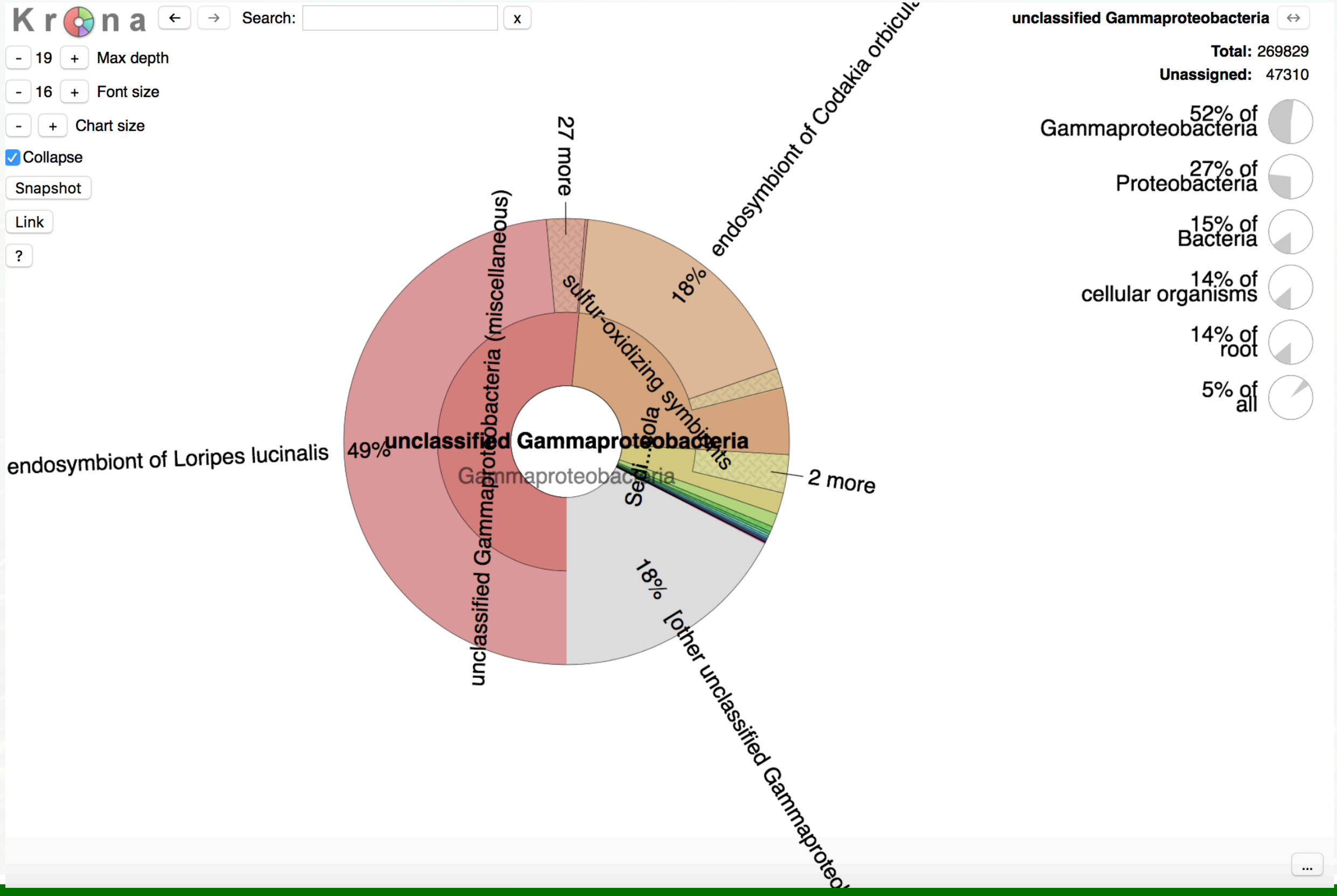
Shotgun Metagenomics Sequencing and Informatics

Kaiju Classification of Reads

Zoomed In


Krona
Search:
x
- 21 + Max depth
- 11 + Font size
- + Chart size
Collapse
Snapshot
Link
?
Gammaproteobacteria
Total: 517171
Unassigned: 66340
51% of Proteobacteria
29% of Bacteria
26% of cellular organisms
26% of root
10% of all
Gammaproteobacteria
Proteobacteria
Bacteria
cellular organisms
endosymbiont of Codakia orbicularis 9%
endosymbiont of Loripes lucinalis 25%
13% [other Gammaproteobacteria]
unclassified Gammaproteobacteria (miscellaneous)
unclassified Gammaproteobacteria
sulfur-oxidizing symbionts
Sedimenticola
Chromatiaceae
Ectothiorhodospiraceae
Oceanospirillaceae
Alteromonadaceae
Pseudomonadaceae
Thiotrichaceae
Piscirickettsiaceae
Cellvibrionaceae
Vibrionaceae
Vibrio
Xanthomonadaceae
16 more
14 more
2 more
27 more
7 more
8 more
248 more
3 more
4 more
58 more
104 more
11 more
x
Legionellales 0.8%
Aeromonadales 0.4%
Pasteurellaceae 0.3%
Nevskiales 0.3%
Acidiferrobacteraceae 0.2%
Orbaceae 0.1%
Salinisphaera 0.07%
environmental samples 0.05%
Cardiobacteriaceae 0.04%

Large #s of “Chemosymbionts” of Clams



Clams in Seagrass Beds

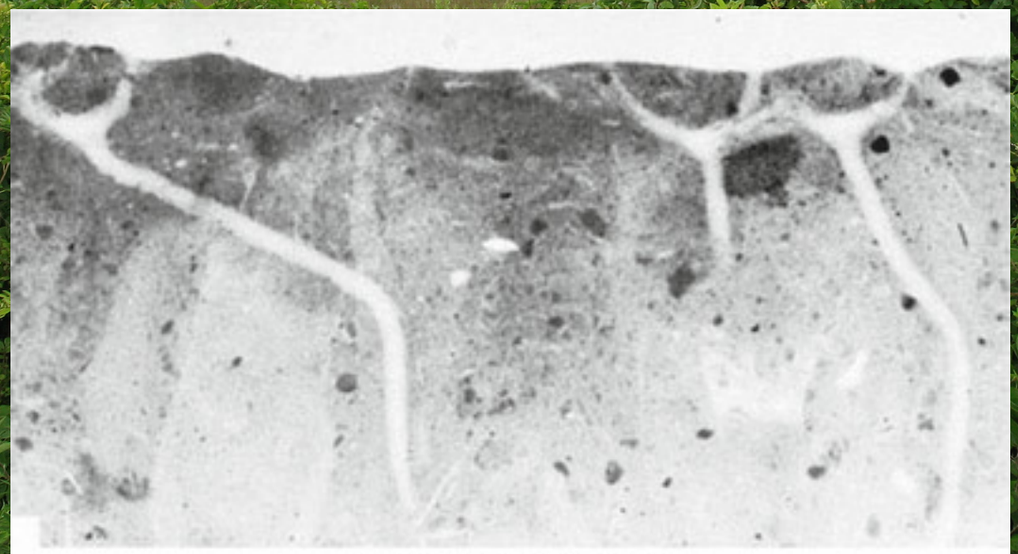


PLATE 3. SOLEMYA

Chemosymbionts of Bivalves

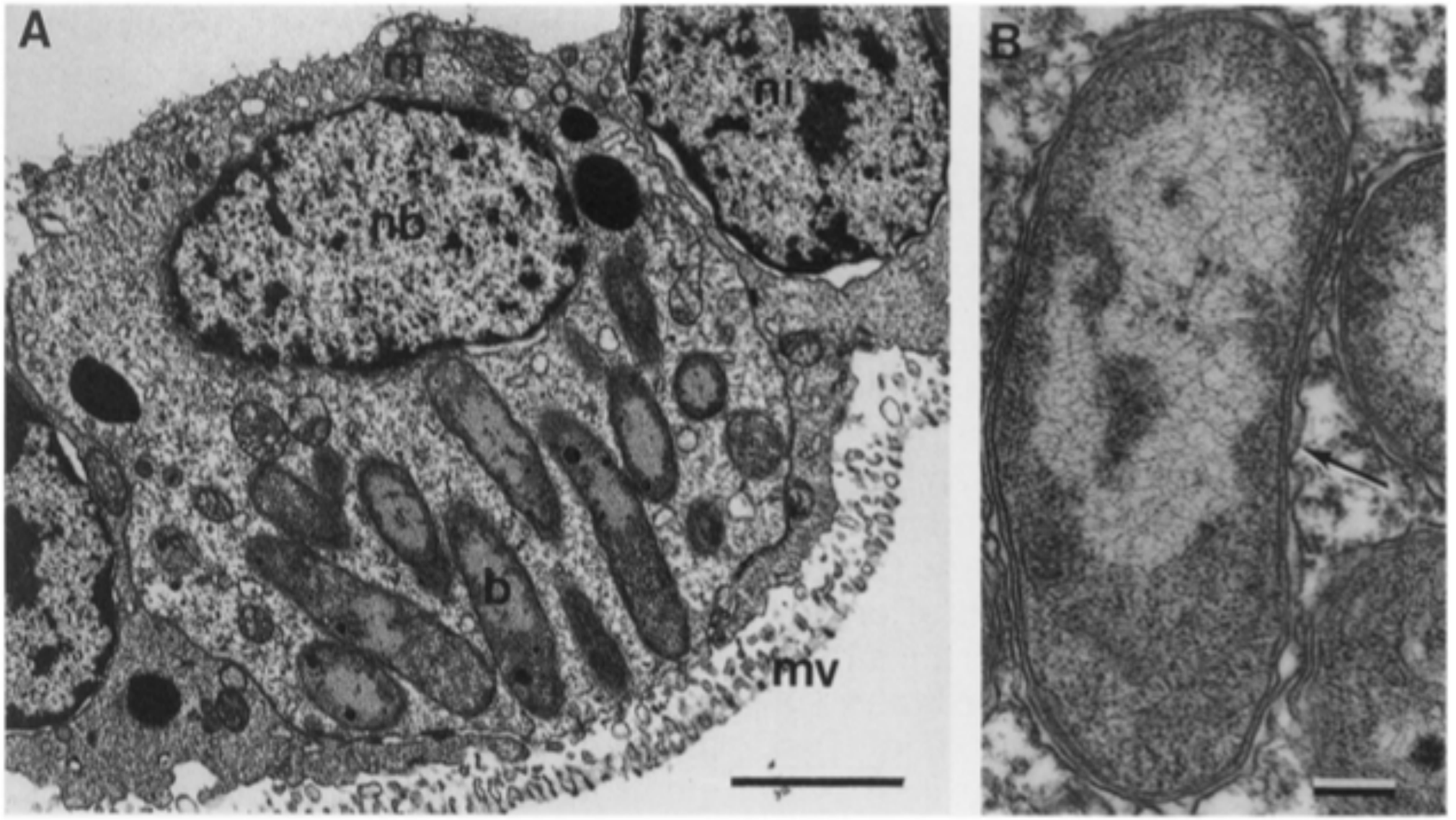
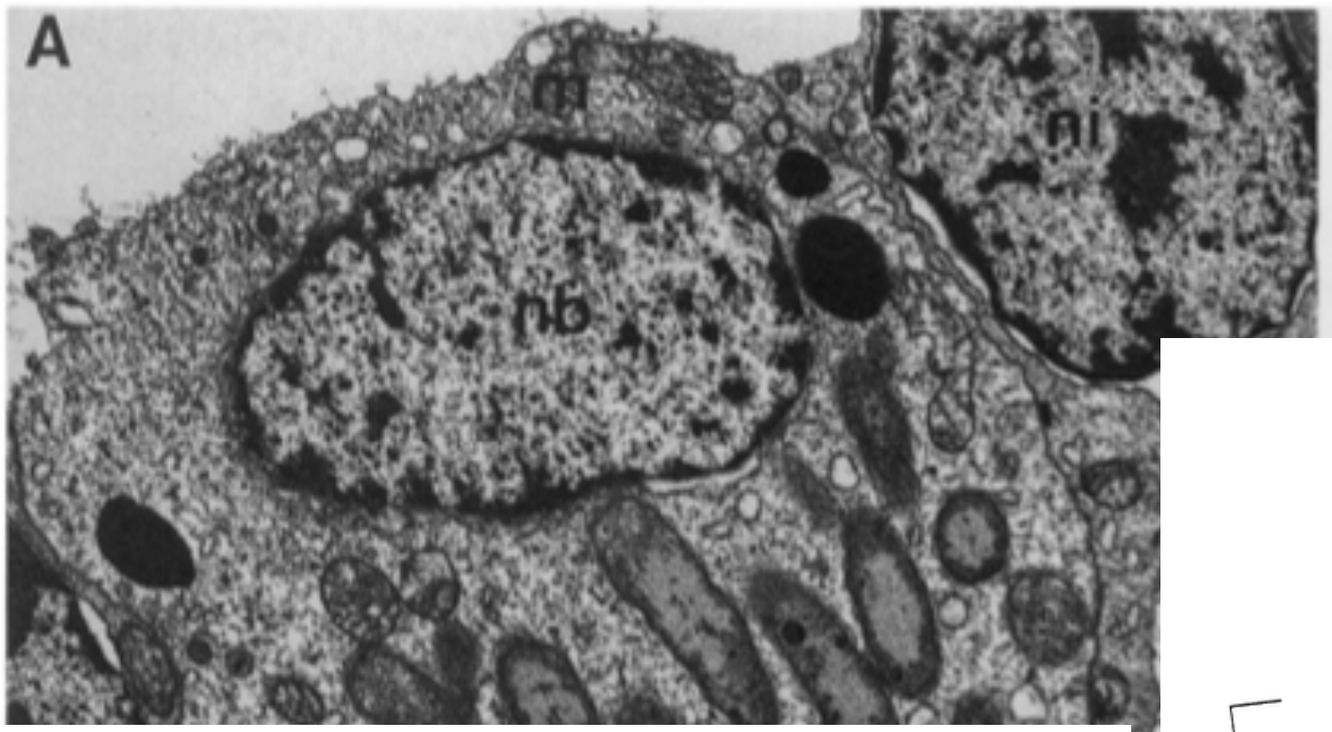


FIG. 2. *Solemya velum*. A. Transmission electron micrograph, transverse section of gill filament, showing rod-shaped bacteria within gill bacteriocyte and intercalary cells lacking symbionts; b: bacteria; mv: microvilli; nb: nucleus of bacteriocyte; ni: nucleus of intercalary cell. B. Same, higher magnification, transverse section of rod-shaped bacterium, showing cell ultrastructure typical of Gram-negative bacteria and peribacterial membrane (arrows). Scale bars, A: 3 μm; B: 0.2 μm. Reprinted with permission from *Biol. Soc. Wash. Bull.* (Cavanaugh, 1985).

Chemosymbionts of Bivalves



JOURNAL OF BACTERIOLOGY, May 1992, p. 3416–3421
0021-9193/92/103416-06$02.00/0

Vol. 174, No. 10

Phylogenetic Relationships of Chemoautotrophic Bacterial Symbionts of *Solemya velum* Say (Mollusca: Bivalvia) Determined by 16S rRNA Gene Sequence Analysis

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Received 4 November 1991/Accepted 9 March 1992

The protobranch bivalve *Solemya velum* Say (Mollusca: Bivalvia) houses chemoautotrophic symbionts intracellularly within its gills. These symbionts were characterized through sequencing of polymerase chain reaction-amplified 16S rRNA coding regions and hybridization of an *Escherichia coli* gene probe to *S. velum* genomic DNA restriction fragments. The symbionts appeared to have only one copy of the 16S rRNA gene. The lack of variability in the 16S sequence and hybridization patterns within and between individual *S. velum* organisms suggested that one species of symbiont is dominant within and specific for this host species. Phylogenetic analysis of the 16S sequences of the symbionts indicates that they lie within the chemoautotrophic cluster of the gamma subdivision of the eubacterial group *Proteobacteria*.

1985).

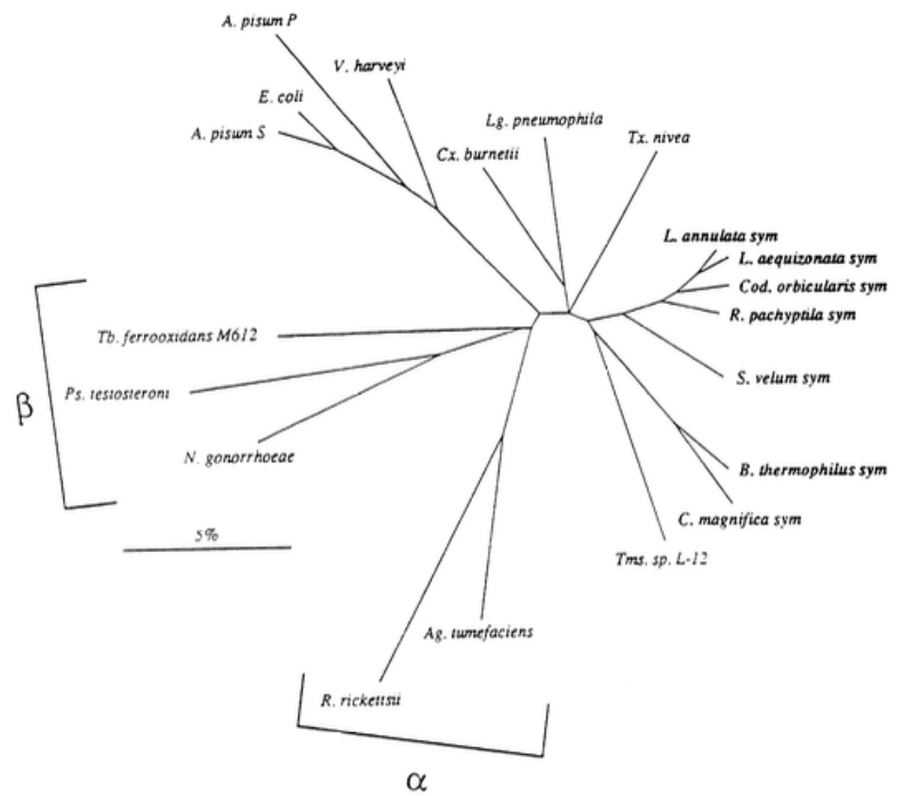
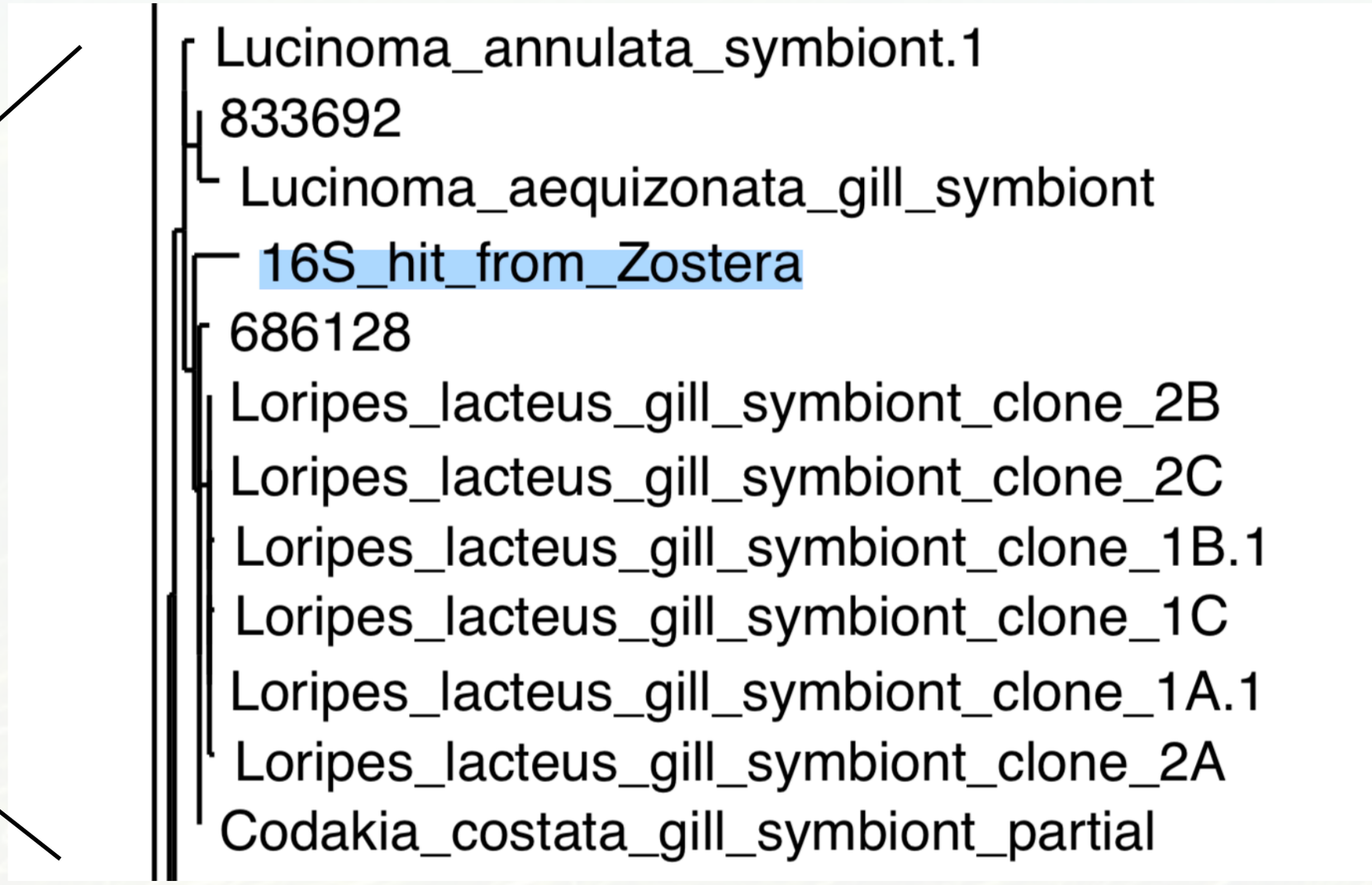


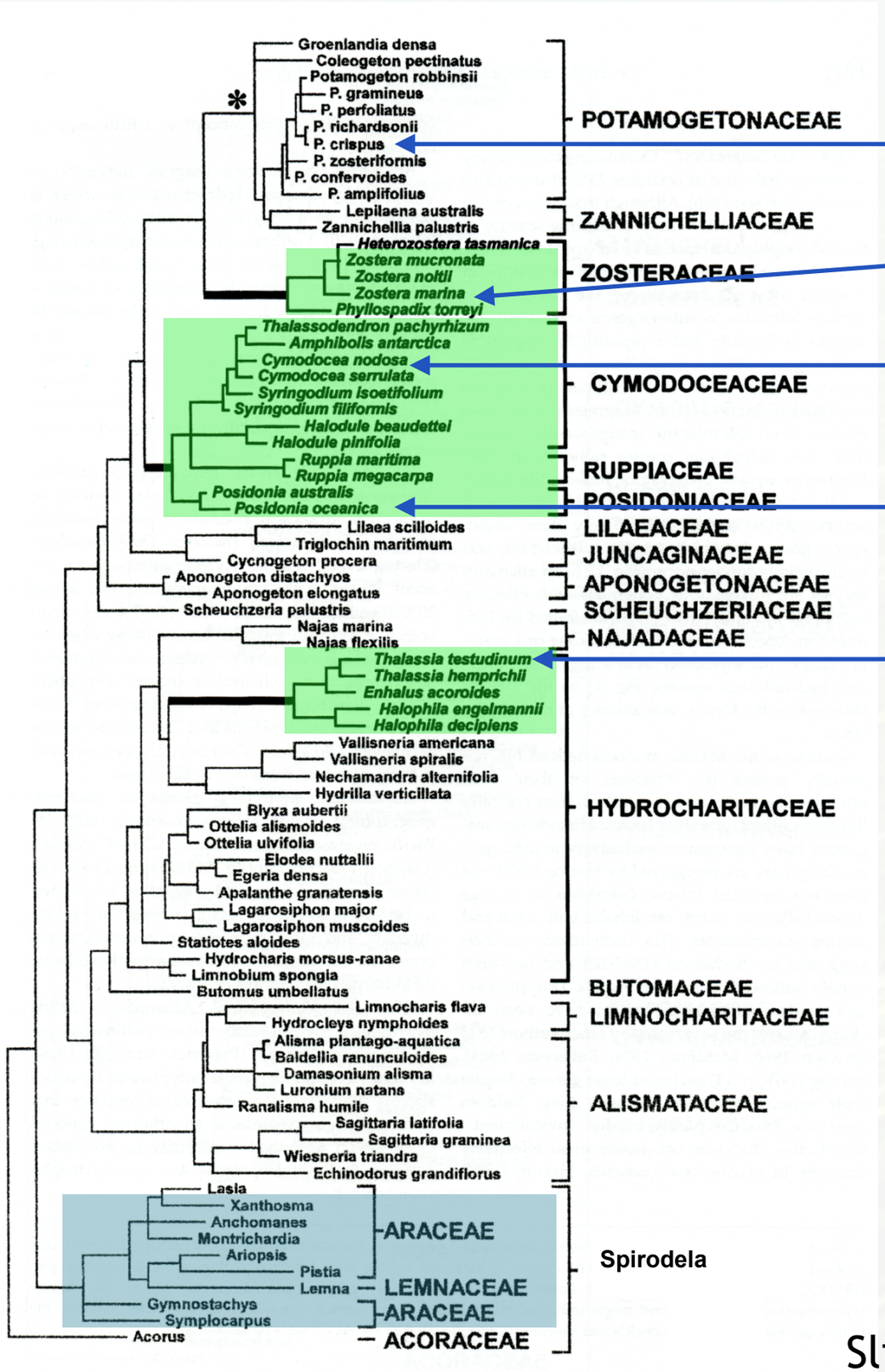
FIG. 4. Unrooted phylogenetic tree, based on evolutionary distances, showing the position of the chemoautotrophic symbionts in relation to that of other Proteobacteria on the basis of 16S rRNA gene sequences. Members of the alpha- (*Rickettsia rickettsii* and *Agrobacterium tumefaciens*) and beta-subclasses (*Thiobacillus ferrooxidans* M612, *Pseudomonas testosteroni*, and *Neisseria gonorrhoeae*) of the Proteobacteria are bracketed; all others are of the gamma subclass. These include chemoautotrophic symbionts (listed in bold type) of bivalves (*Lucinoma aequizonata* sym, *L. annulata* sym, *Codakia orbicularis* sym, *Solemya velum* sym, *Bathymodiolus thermophilus* sym, *Calyptogena magnifica* sym) and the vent tubeworm (*Riftia pachyptila* sym), free-living species (*Thiomicrospira* sp. L-12, *Thiothrix nivea*, *Legionella pneumophila*, *Coxiella burnetii*, *Escherichia coli*, and *Vibrio harveyi*), and aphid symbionts, *Acyrthosiophon pisum* S and *Acyrthosiophon pisum* P. Sym = symbiont. Reprinted (with modifications) from *Journal of Bacteriology* (Eisen *et al.*, 1992).

Chemosymbionts



Future Plans

JGI Seagrass Pop Geno/Microbiomics



Potamogeton crispus

216 *Zostera marina*

Cymodocea nodosa

Posidonia oceanica

Thalassia testudinum

JGI JOINT GENOME INSTITUTE DEPARTMENT OF ENERGY

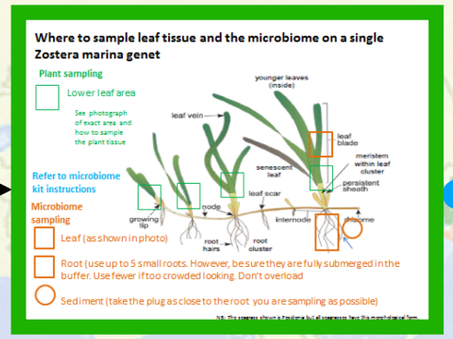
Jay Stachowicz

Jeanine Olsen

Yves van De Peer

Slide by Laura Vann from Tree from Les et al., Syst. Bot. 1997

JGI Seagrass Population Sampling



- Sent kits
- Sampled microbiomes of leaves, roots, sediment
- Sampled leaves for genomes

ZEN
Zostera Experimental Network

http://zenscience.org

Jay Stachowicz

Jeanine Olsen

Laura Vann

Microbial Manipulation of Seagrass?

Raquel Peixoto

Microbial Manipulation of Mangroves

Raquel Peixoto

Before… no plants even after revegetation

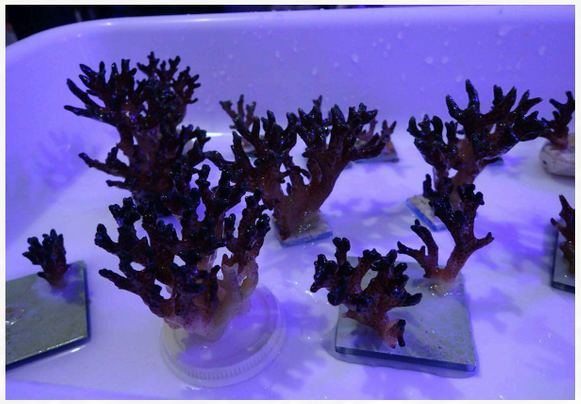
3 years after PGPR oil degrading + biostimulation treatment

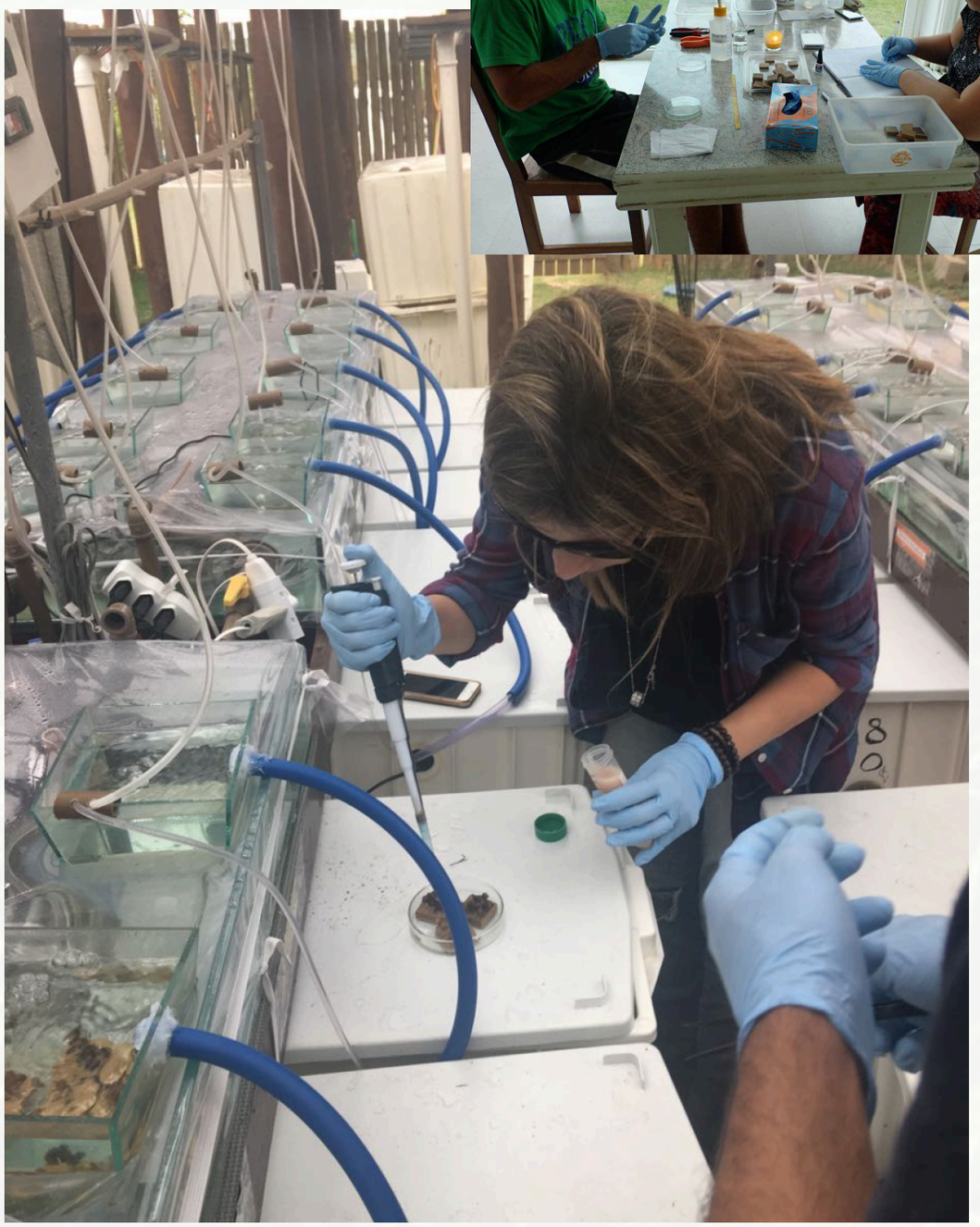
Taketani et al., 2009 (Journal of Microbiology), 2010 (Antonie); Santos et al., 2010 (PLOS One), Santos et al., 2011 (PLOS One); Santos et al 2010 WASP; Peixoto et al., 2011 (Antonie) Carmo et al., 2011 TJOM; Peixoto et al., *in preparation;*
Carmo, Santos, Peixoto, Rosado, patent 2016

Microbial Manipulation of Coral

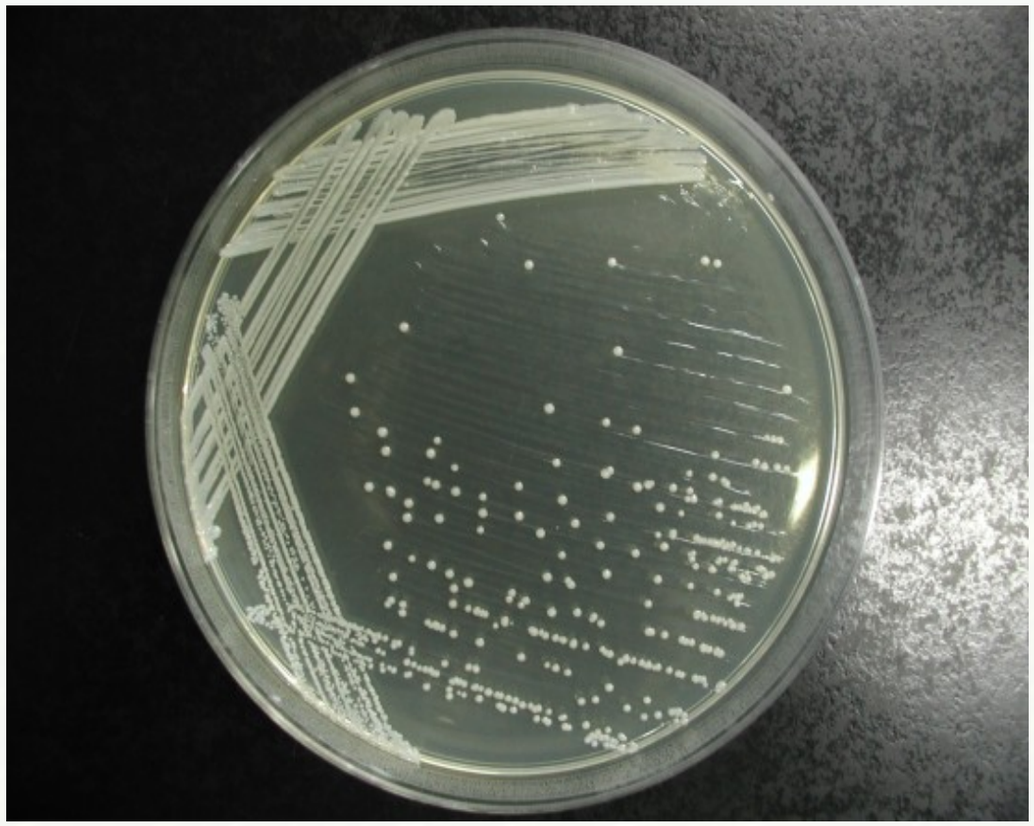
Probiotic consortium from *Pocillopora damicornis*

BMC screening
7 strains





Microbial Manipulation of Seagrass?



Jay Stachowicz

Melissa Kardish

Raquel Peixoto

Massively Parallel Undergraduates

- Karley Lujuan
- Marcus Cohen
- Katie Somers
- Taylor Tucker
- Hoon San Ong
- Neil Brambhatt
- Hena Hundal
- Daniel Oberbauer
- Briana Pompa-Hogan
- Alex Alexiev
- Ruth Lee

Pic of Karley Lujuan

David Coil

Seagrass Microbiome Project
Key Lesson:

Good People Are A Good Thing

Guillaume Jospin

Alana Firl

Laura Reynolds

Katie DuBois

Jessica Abbott

Susan Williams

James Doyle

Russell Neches

Melissa Kardish

Cassie Ettinger

Sofie Voerman

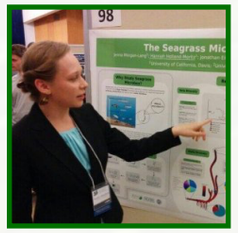
Yves van De Peer

Jeanine Olsen

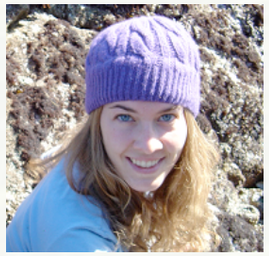
Laura Vann

Ashkaan Fahimipour

Ruth Lee



Hannah Holland-Moritz



Pamela Reynolds

ZEN

Zostera Experimental Network

Jay Stachowicz

Jessica Green

Jenna Lang

David Coil

- Karley Lujuan
- Marcus Cohen
- Katie Somers
- Taylor Tucker
- Hoon San Ong
- Neil Brambhatt
- Hena Hundal
- Daniel Oberbauer
- Briana Pompa-Hogan
- Alex Alexiev
- Ruth Lee

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