



Shark Bay microbialites: a walk through technological time

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Pieter Visscher

DEC symposium on research and
conservation

October 2012



Outline

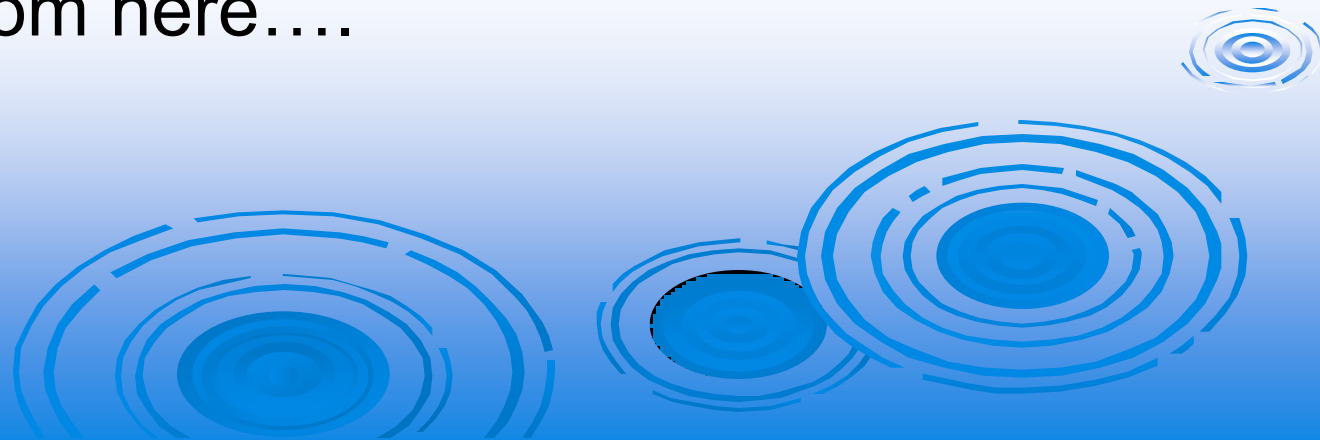
1. Shark Bay microbialites
2. Initial diversity studies
3. Tagged pyrosequencing
4. Functional metagenomic sequencing
5. Microelectrode profiling
6. Conservation and sustainability
7. Where to from here....

Applications of genomic, proteomic, microbiological, and analytical chemical tools in the study of functional complexity



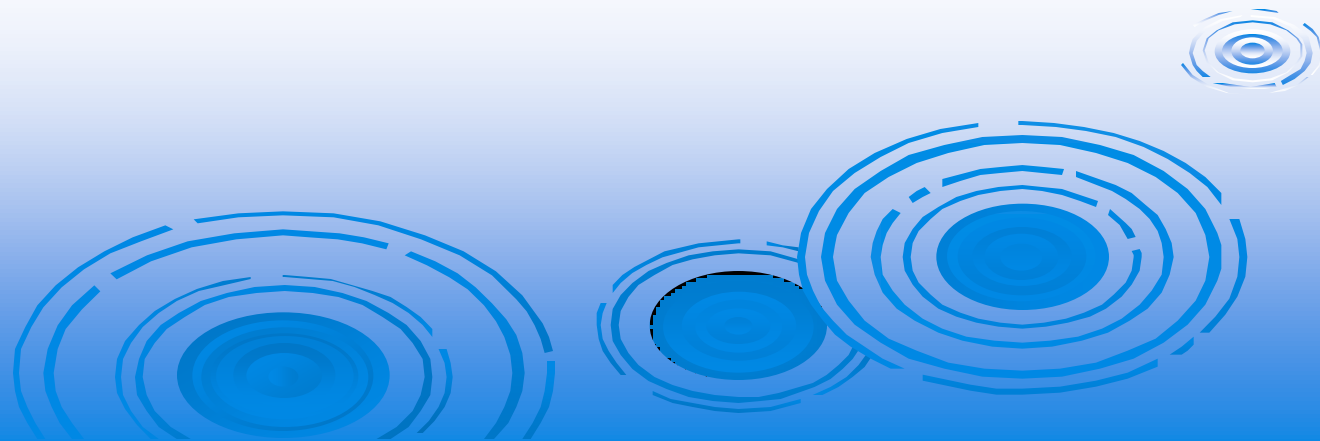
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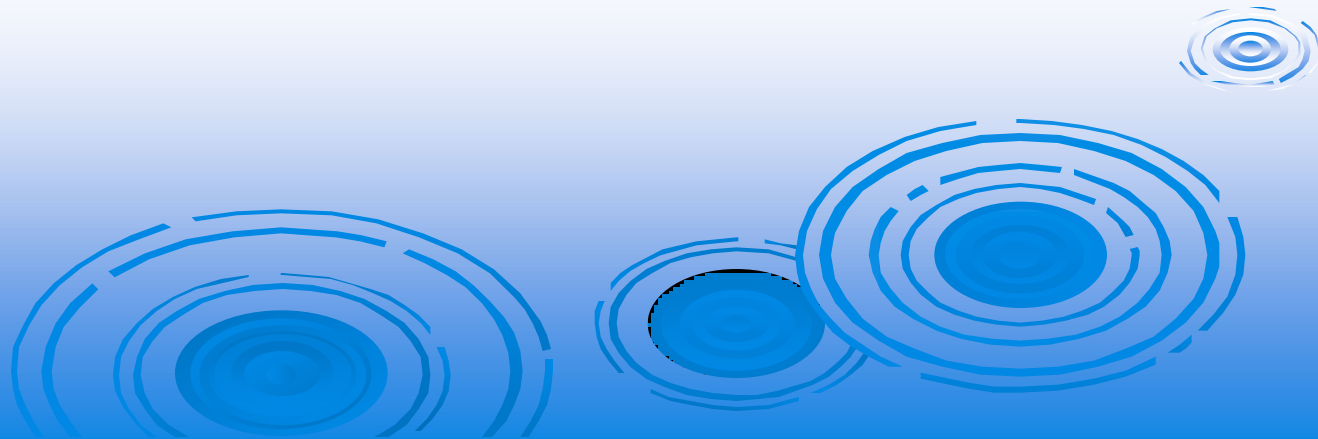
Overall strategic research goals

Comprehensively study the functional complexity of modern microbialites, as a model system for understanding the intricate interactions between microorganisms and their physical environment and their potential roles in the wider biosphere



Hypothesis

Microbial metabolisms (and interactions)
determine stromatolite function, morphology, and
persistence



Hamelin Pool (Shark Bay) ,Western Australia



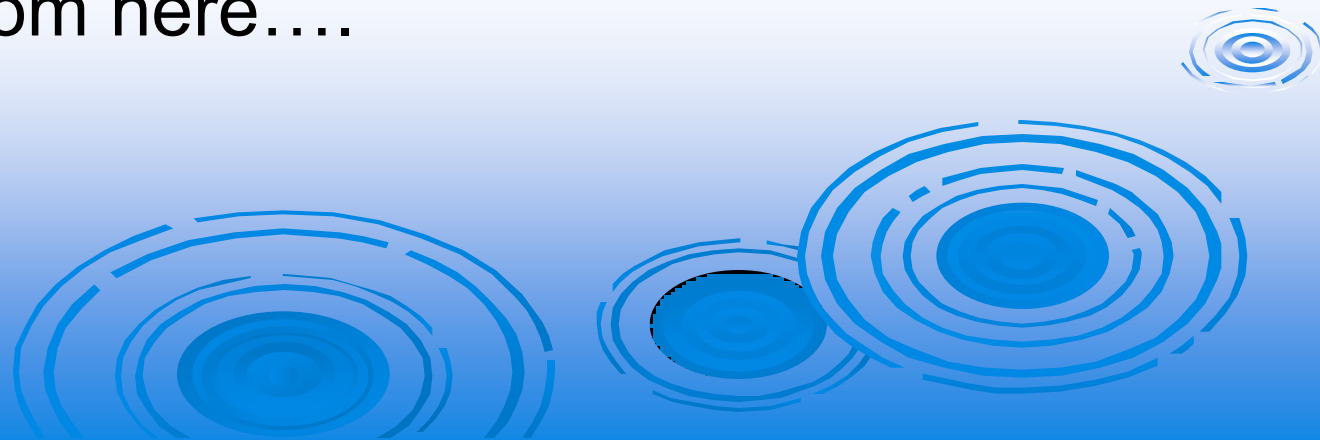
Shark Bay microbialites

- One of the best examples on earth of living marine microbialites
- Surrounding seawater at least twice as saline as normal seawater (fluctuates); high UV, desiccation
- Higher salinity in early oceans
- Key to understanding the past is to study the present



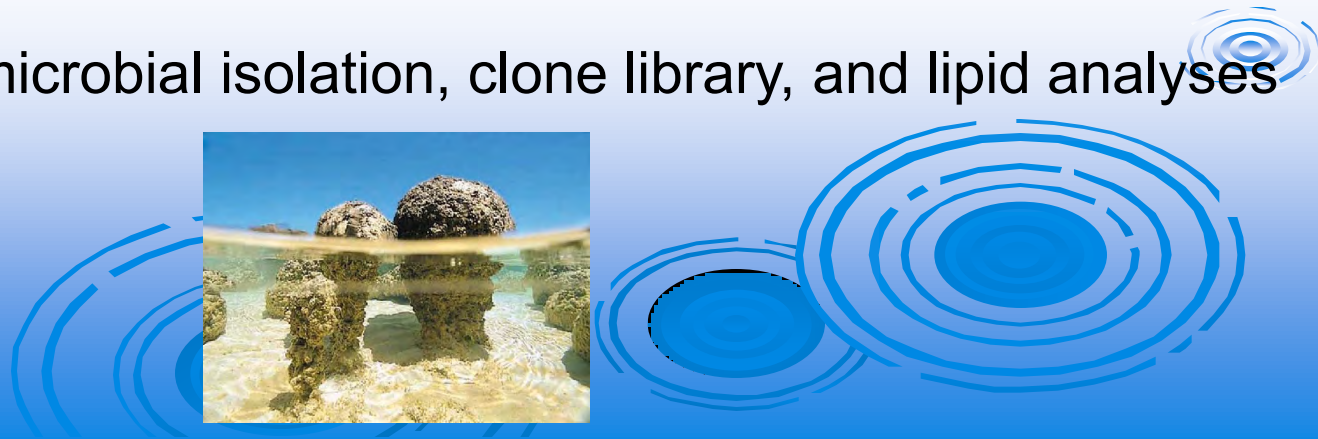
Outline

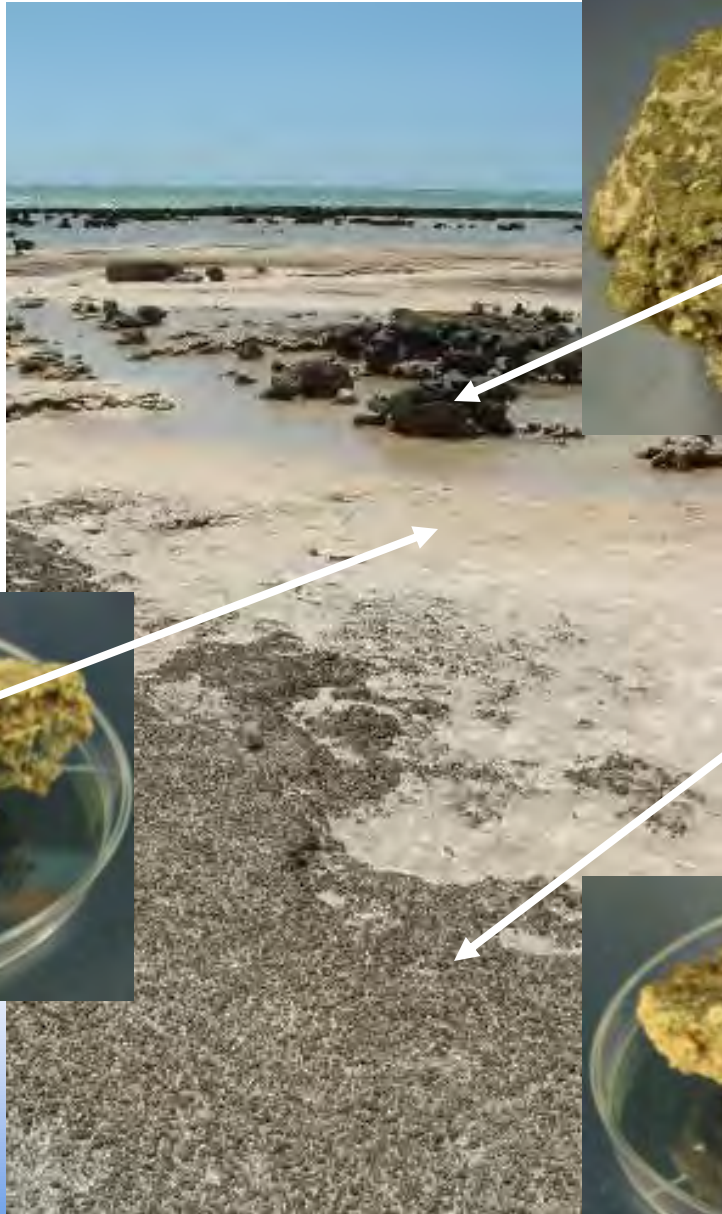
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Initial research goals

- Little was known about specific associated microbial communities
- Earlier studies focused on morphological analyses (*Entophysalis* sp., *Microcoleus* sp.)
- Characterising microbial diversity critical first step
- First goal to undertake comprehensive survey of microbial populations in living microbialites of Shark Bay WA
- Combination of microbial isolation, clone library, and lipid analyses





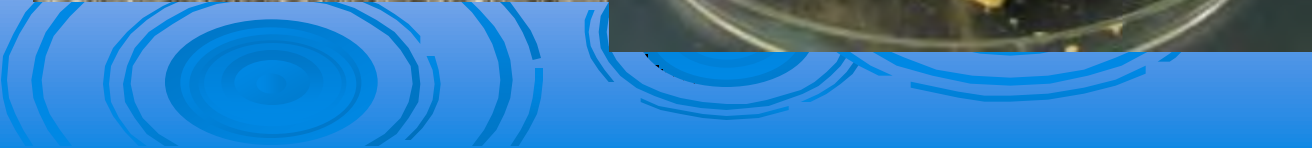
Smooth Mats



Stromatolites



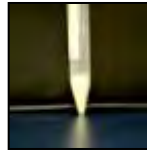
Pustular Mats



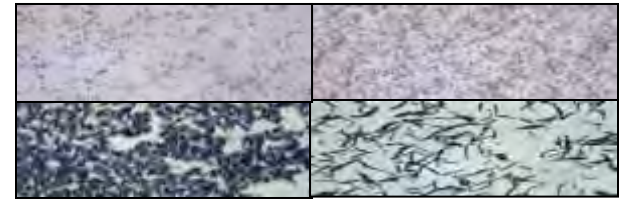
Initial aims

- What are the microbial populations of the Hamelin Pool intertidal microbialites?
- What (if any) correlations exist between the populations of the mats and the stromatolites in the intertidal zone?
- What lipid biomarkers characterise these communities?
- Are these microbialites good analogues for early life on Earth as recorded in ancient sediments?

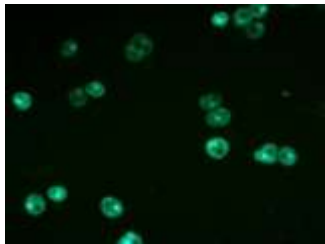
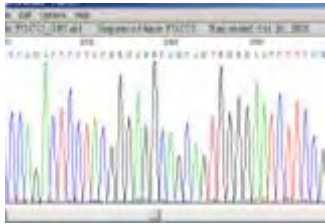




microorganism
isolation

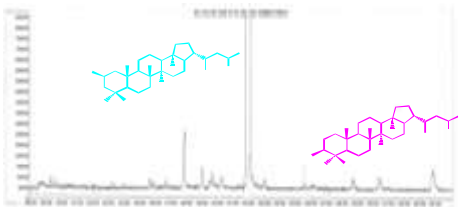
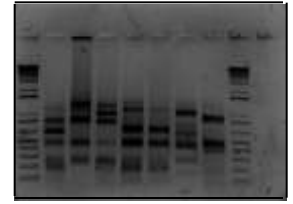
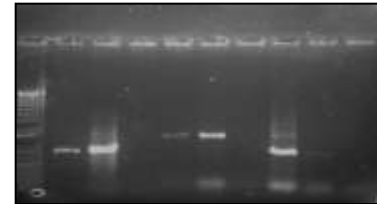


Total DNA extraction;
clone library
construction



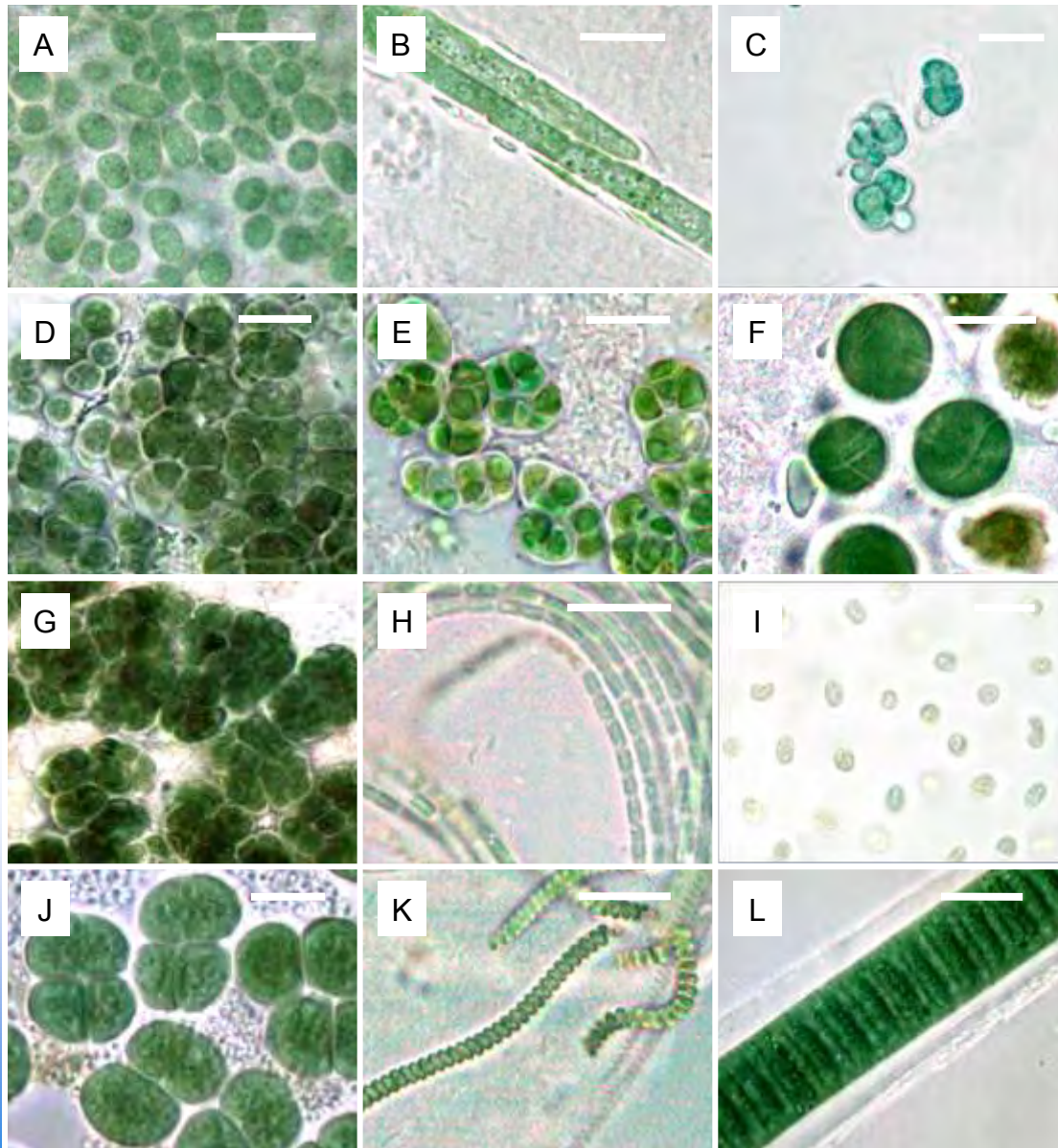
molecular analyses
(DNA extraction, PCR, sequencing)

functional
analyses

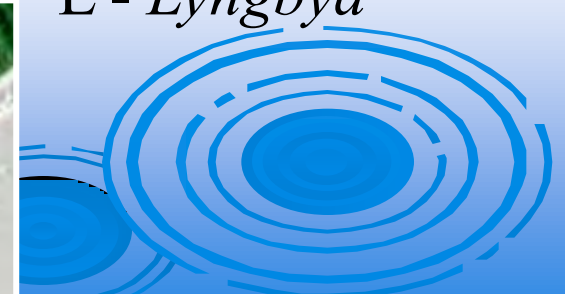


Care needed when inferring function from phylogeny

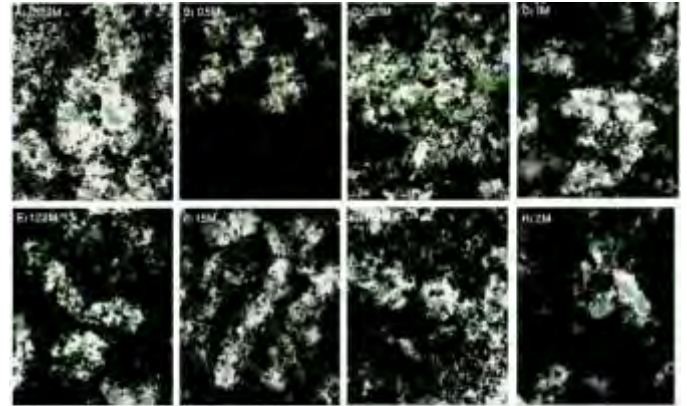
Cultured cyanobacterial isolates



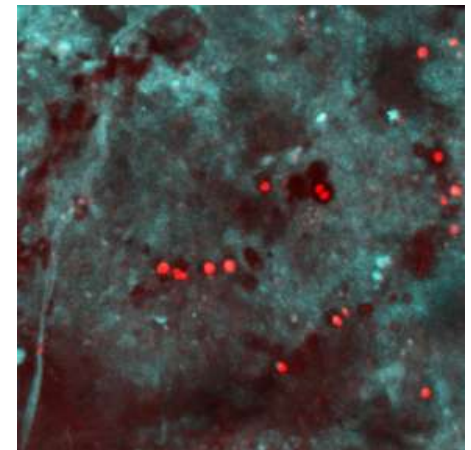
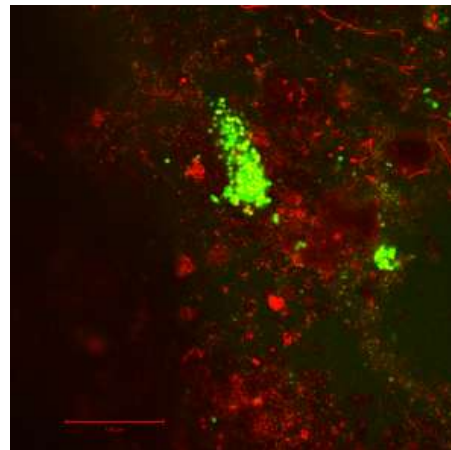
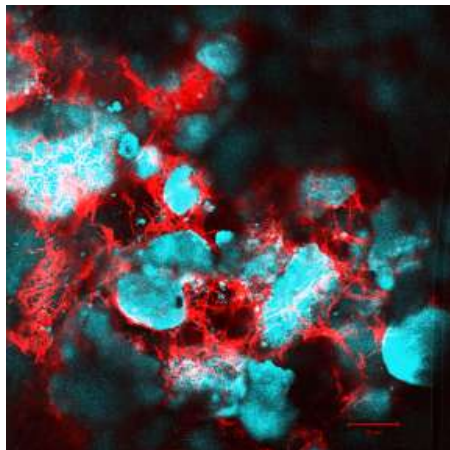
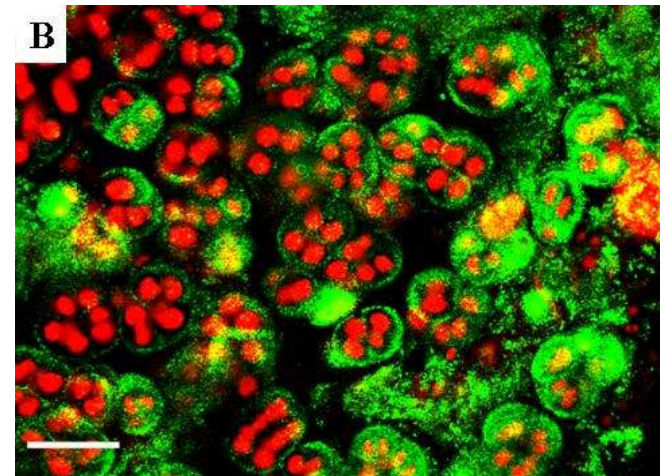
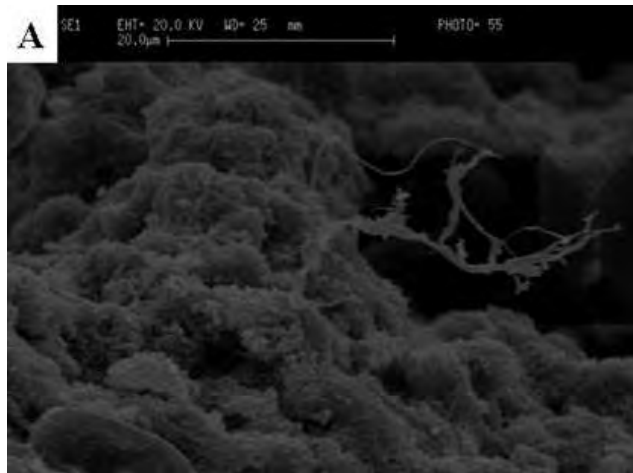
- A - *Eukalythoece*
- B - *Microcoleus*
- C - *Cyanothece*
- D - *Myxosarcina*
- E - *Stanieria*
- F - *Chroococcidiopsis*
- G - *Stanieria*
- H - *Halomicronema*
- I - *Halothece*
- J - *Chroococcus*
- K - *Spirulina*
- L - *Lyngbya*



Stromatolites forming in the lab....

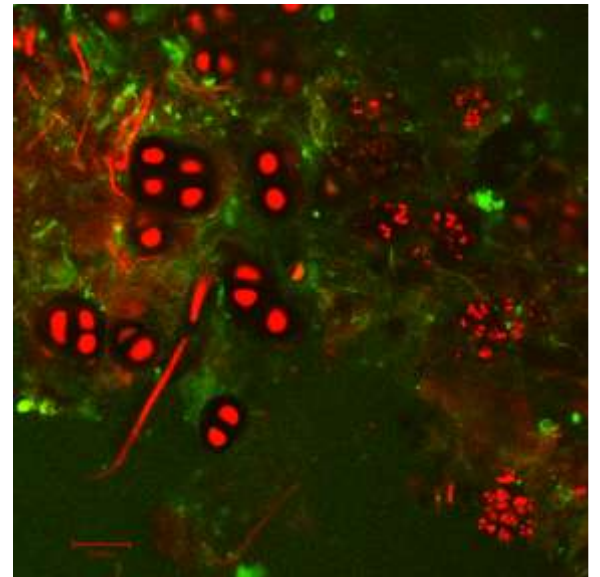
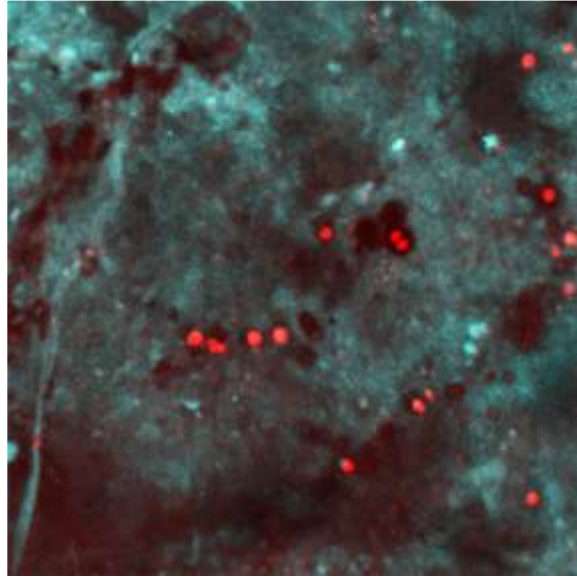
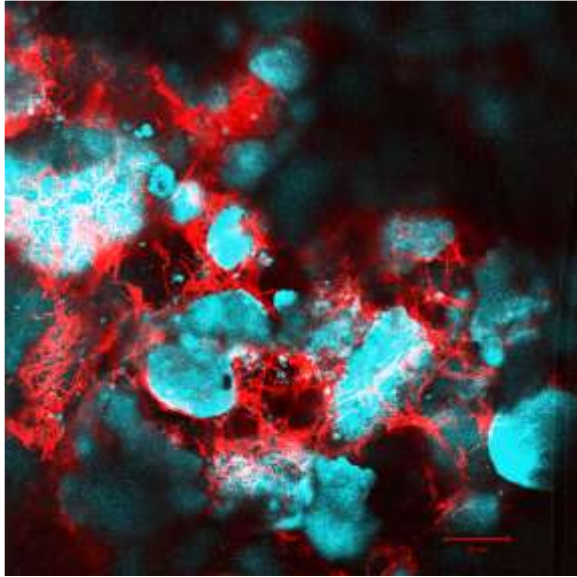


Cell-cell interactions in microbialites

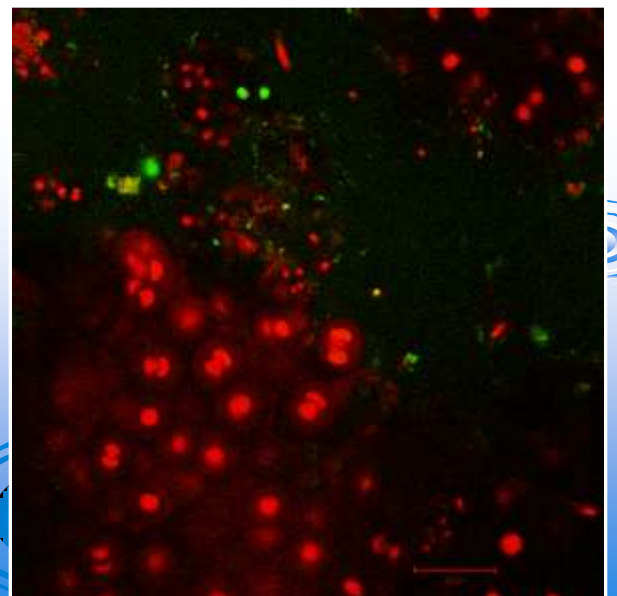
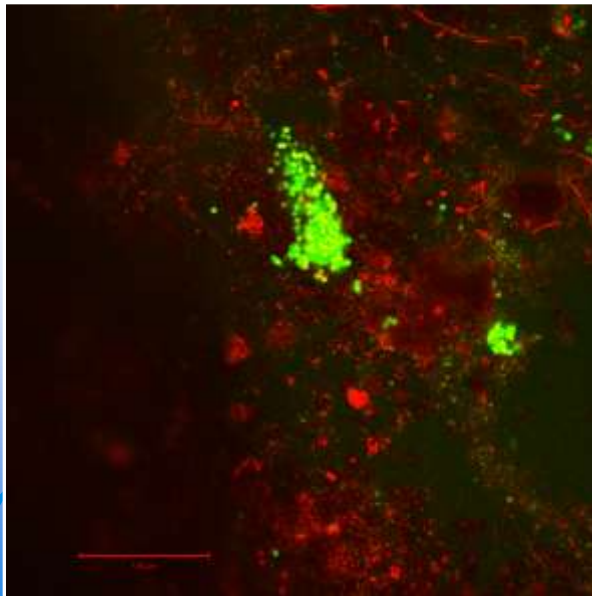
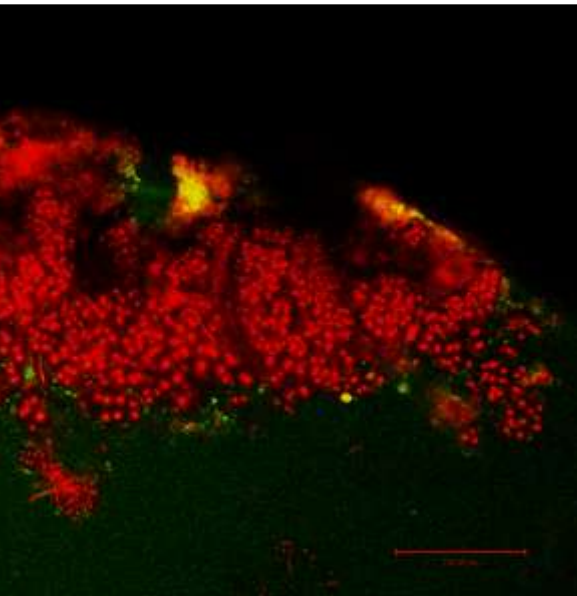


Confocal microscopy

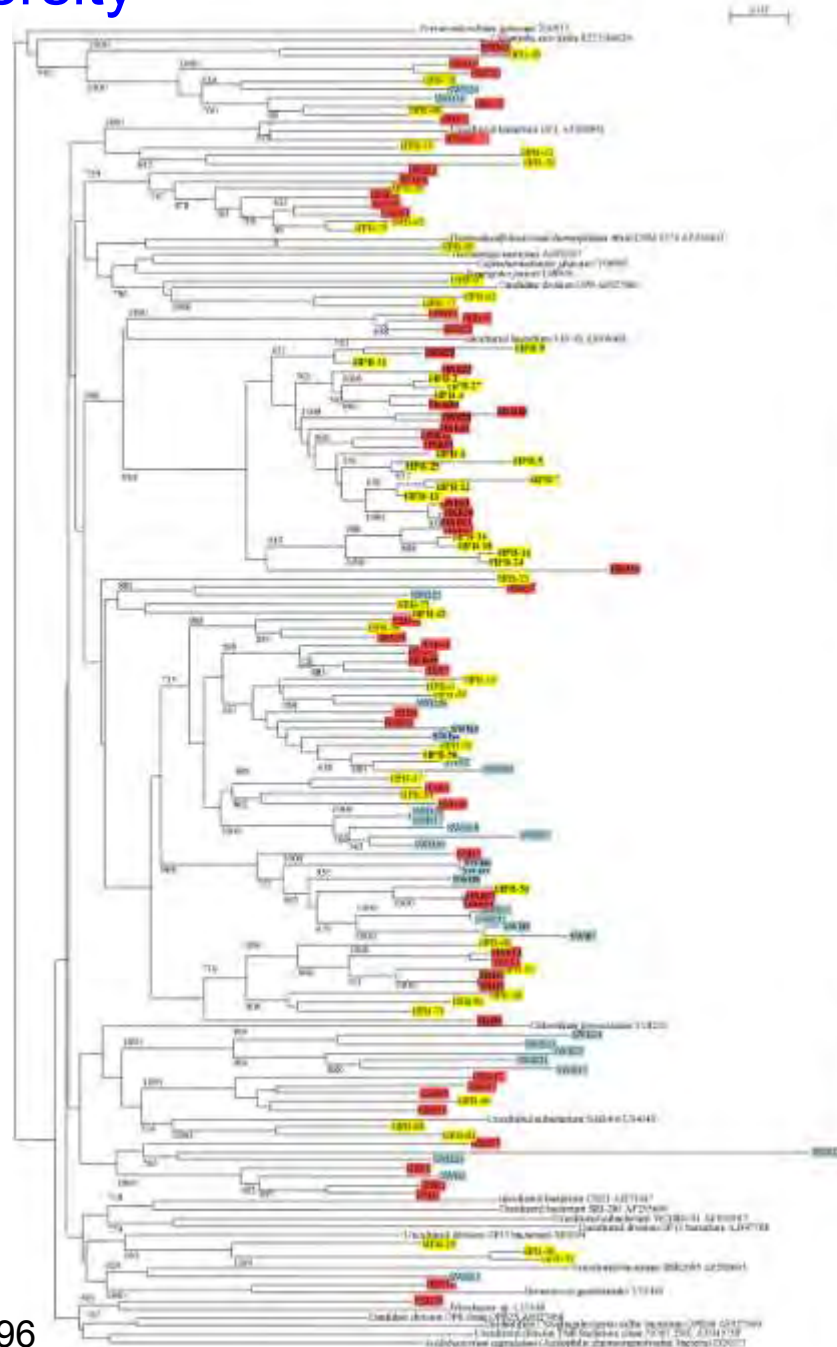
Smooth Mat



Pustular Mat



Total bacterial diversity



Planctomycetes

Unknown group

Actinobacteria

OP-9 Group

Firmicutes/ Gram +

Proteobacter
Purple Bacteria

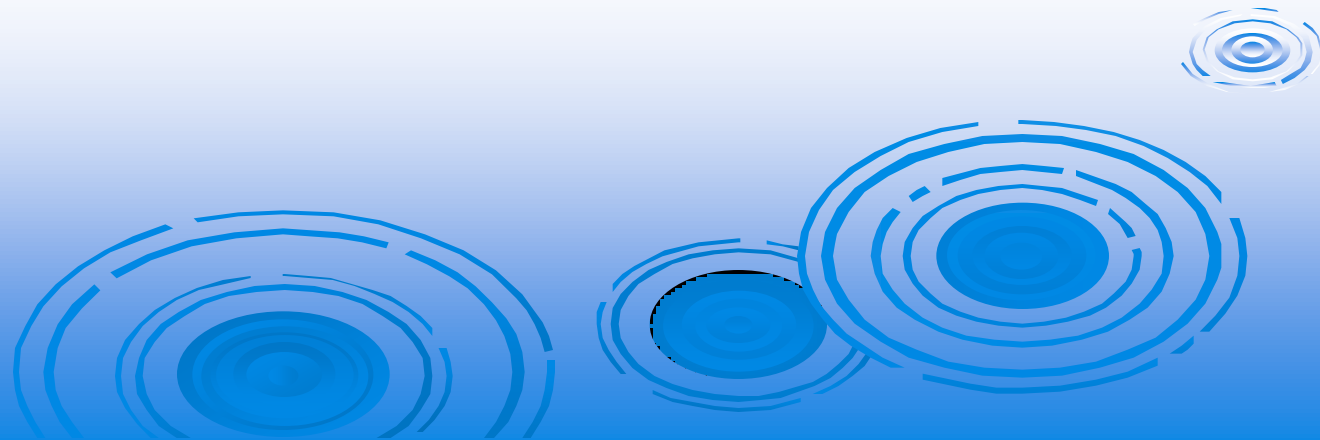
Green sulfur bacteria

Cyanobacteria

Fibrobacter

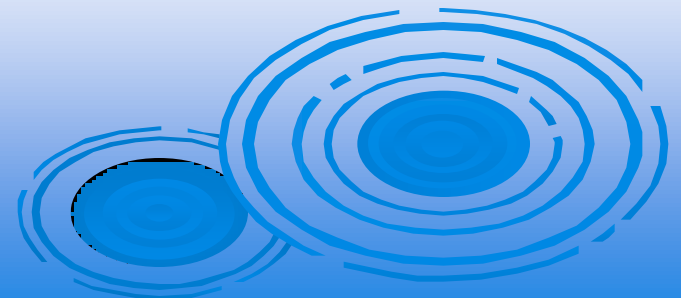
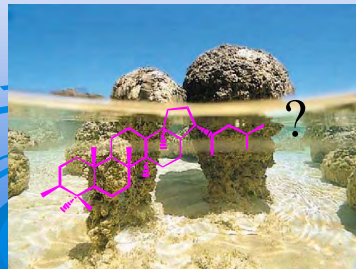
So with this understanding of the microbial populations of Shark Bay microbialites...

What lipid biomarkers do these communities produce?



Lipid biosignatures

- Lipids have numerous physiological functions
- Serve as biomarkers or chemical fossils - indicators of biogenic, paleoenvironmental, and geochemical processes
- Carbon skeletons survive over billions of years
- Lipid biomarkers can (potentially) establish connections between modern microbial ecosystems and their ancient counterparts
 - Eg, 2-methyl-bacteriohopanoids in cyanobacteria

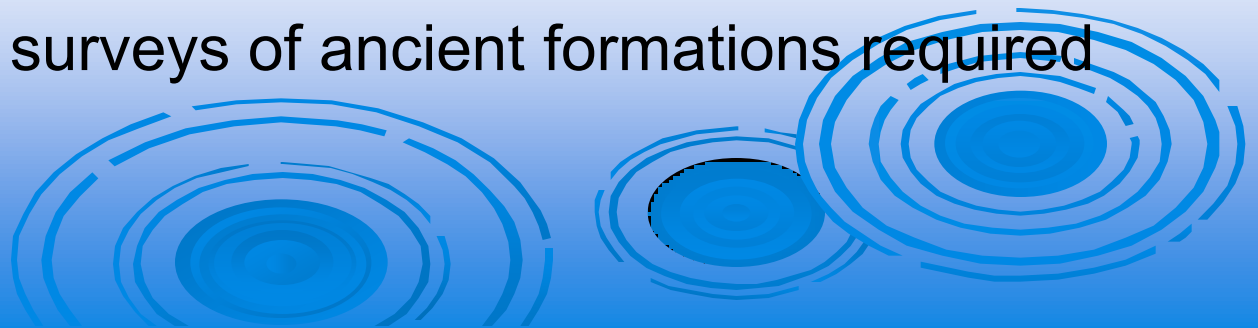


Lipid analyses - summary

- FAME, hydrocarbon, hopanoid - oxygenic photosynthesis
- FAME, wax esters - anoxygenic phototrophs
- FAME, wax esters, hydrocarbon - heterotrophic metabolism
- FAME, BACQ - sulphur cycling
- Ether bound phytane - archaea
- Sterols - bivalves and their dinoflagellate symbionts

- Functional groups of organisms detected by signature lipids correlated with metabolisms inferred from 16S rDNA

- More extensive surveys of ancient formations required

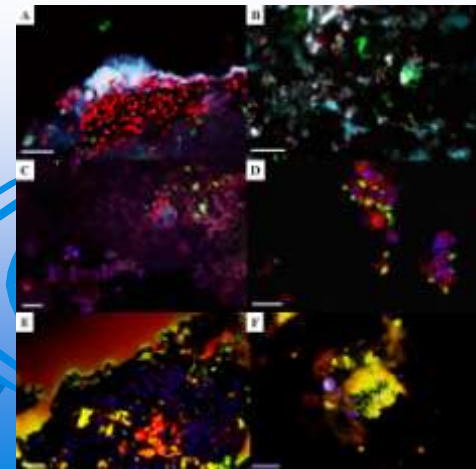


Diversity analyses - methods overview

Method:	Isolation	Cloning 16S rDNA	Lipid biomarkers
Target:	Living population	Total population	Total population, past and present
Pos:	Isolates available for further study	Ubiquitous Large databases	Ubiquitous Very stable Some are specific
Neg:	Time consuming Unculturable	Multiple copies in cell	Time consuming

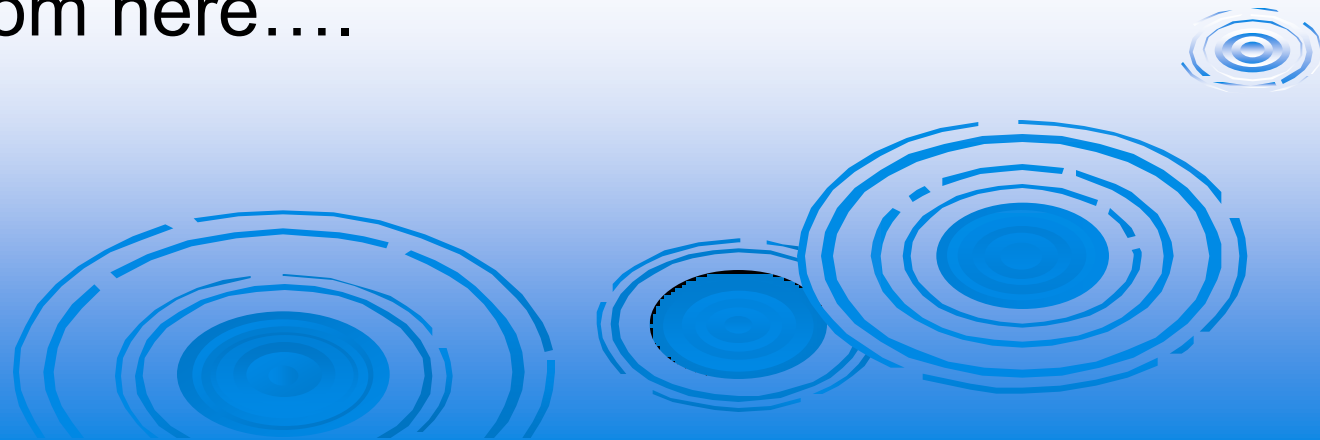
Microbialite microbial diversity

- First detailed study of microbialite microbial communities
- Supports range of metabolically and phylogenetically diverse microbes - distinct communities in different mat types
- Key functional groups - high level of biological complexity
- Many unknown, potentially unique organisms
- Use these phylogenetic inferences to rationally characterise functional complexity:
 - Target specific functional genes/proteins
 - Characterise specific organisms/interactions
 - Study stress responses
 - Examine biomarkers
 - Cell communication



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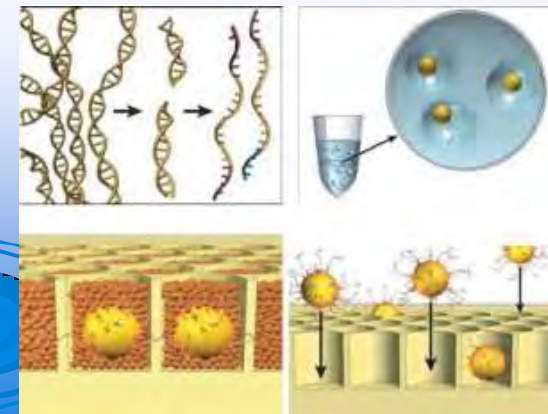
High throughput tagged pyrosequencing

- Taxonomic assessment of stromatolite communities via barcoded pyrosequencing
- Allows for deep population assessment
- 454 (Roche) pyrosequencing technology
- Results in large data sets and costs coming down

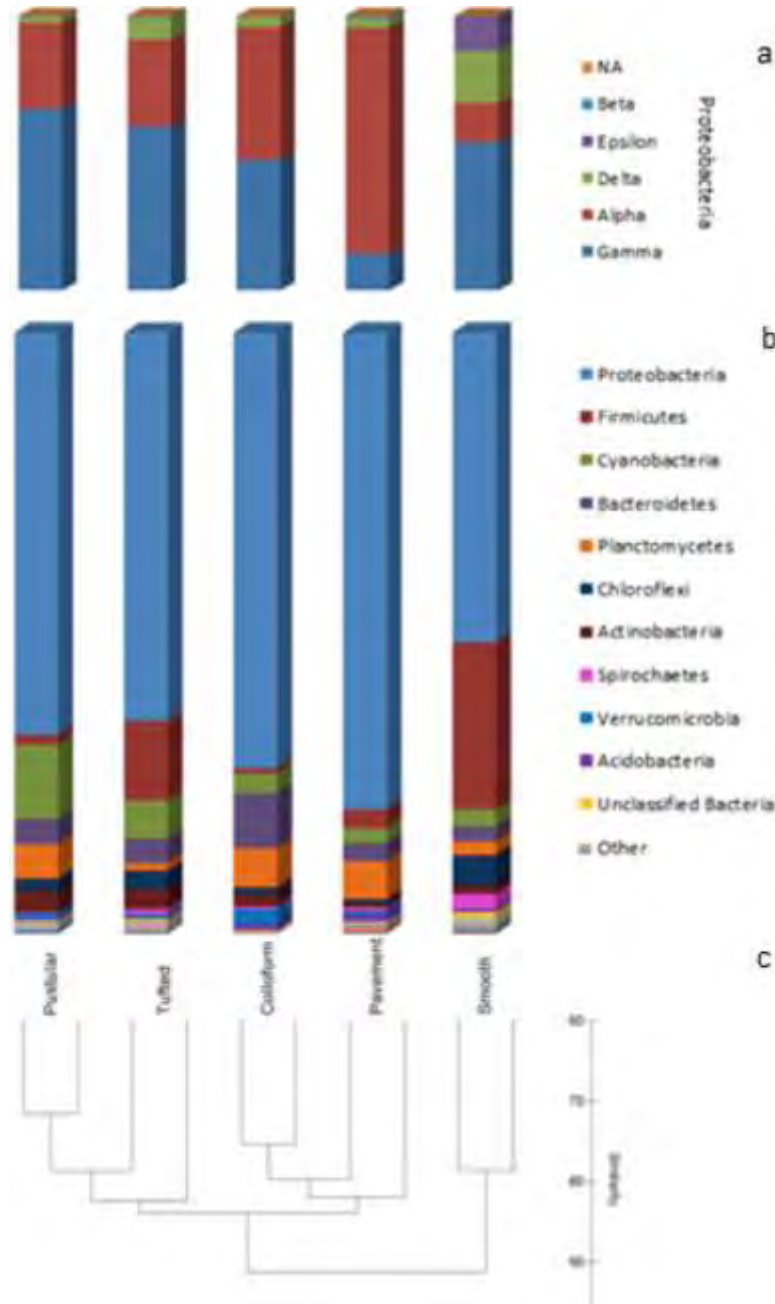


Tagged pyrosequencing of Shark Bay microbialites

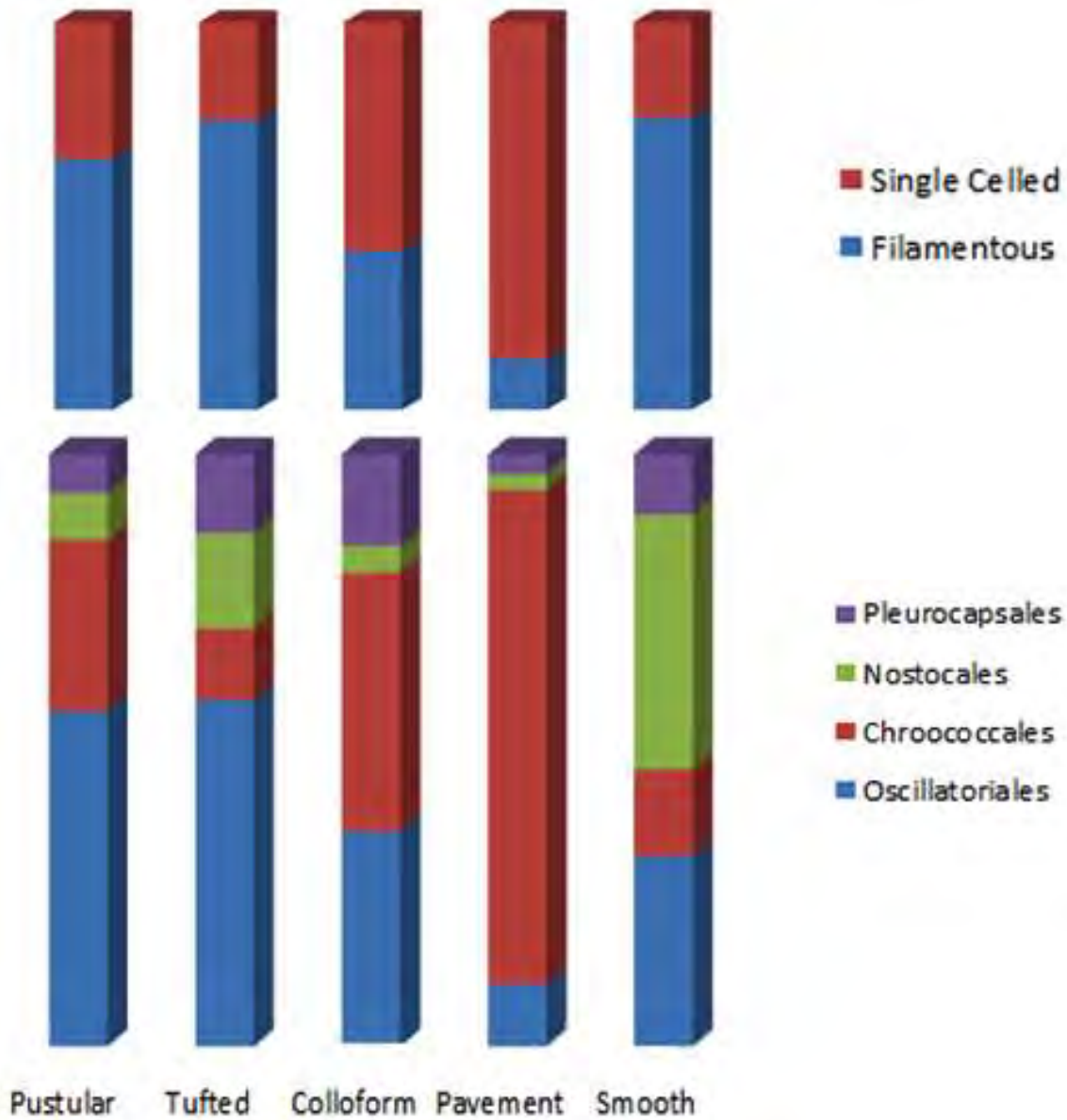
- Extraction of total DNA from microbialites
- Quality control test with 16S rDNA bacterial and archaeal primer sets
- Whole DNA sequencing and 16S rDNA tagging with archaeal and bacterial primers
- Data analysis



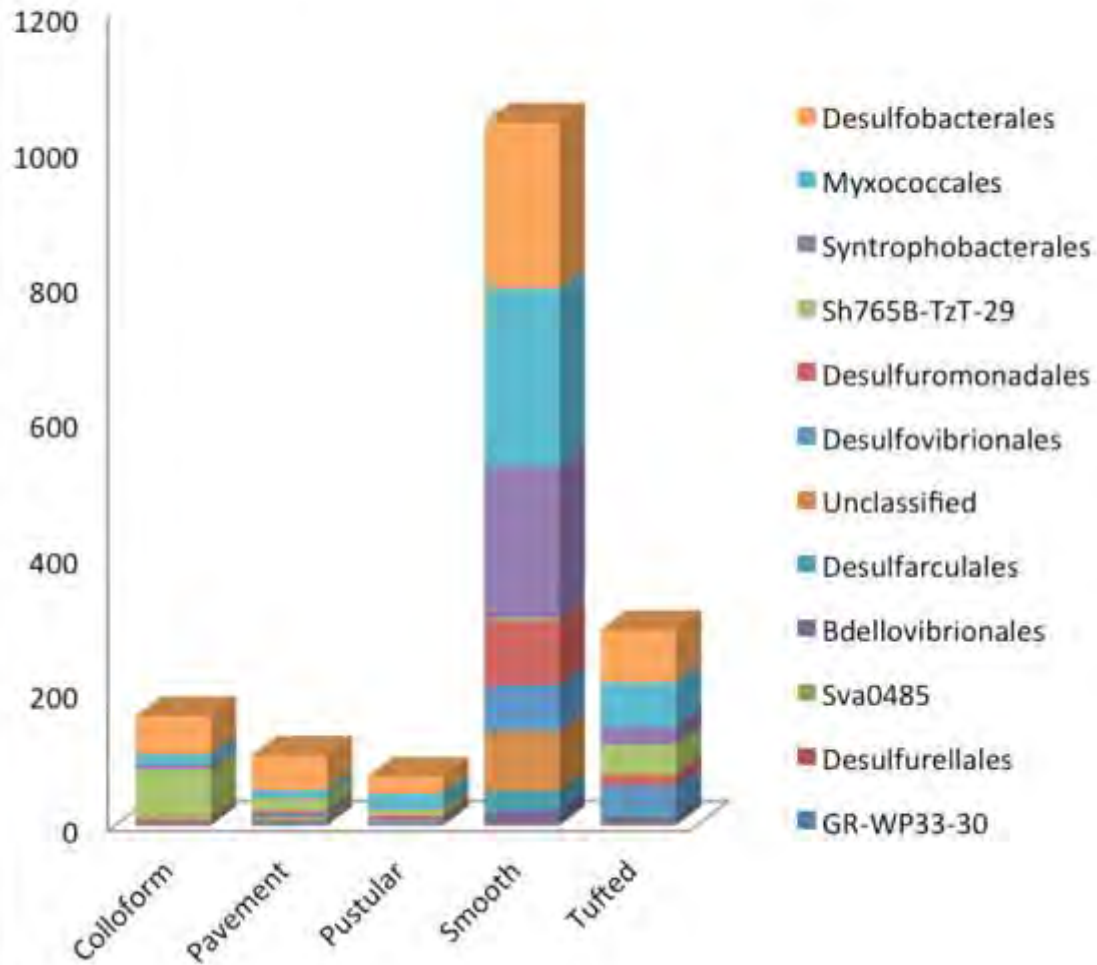
Diversity among mat types



Cyanobacterial morphotypes

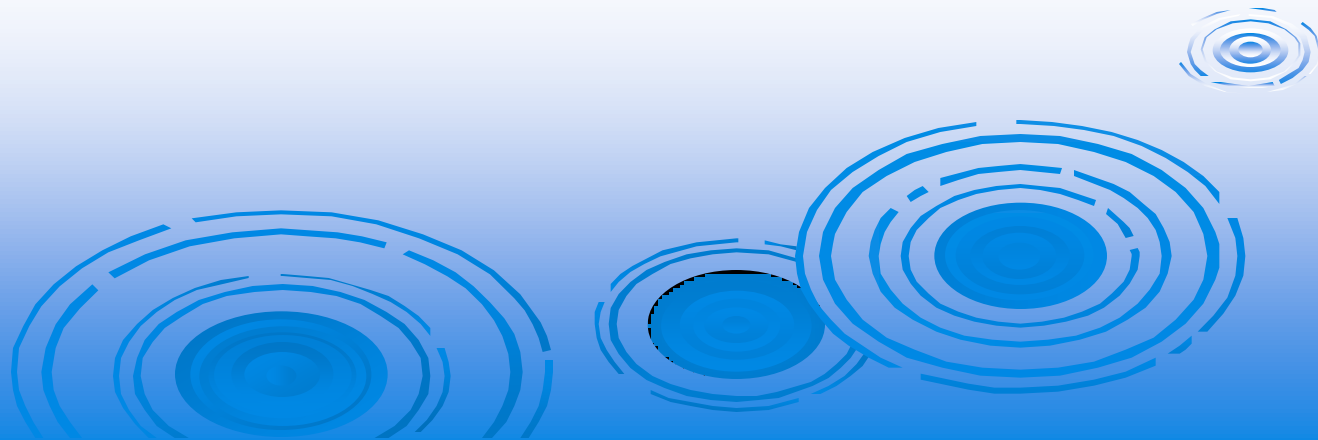


Deltaproteobacteria



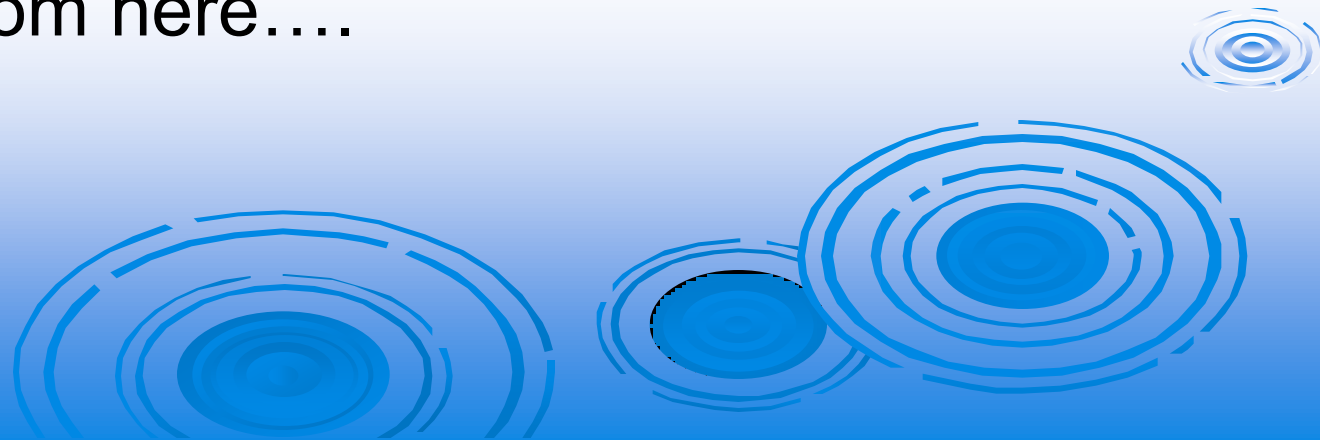
So this tells us about microbial diversity of Shark Bay microbialites but...

Only limited conclusions can be made regarding functional metabolic potential



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4. **Functional metagenomic sequencing**
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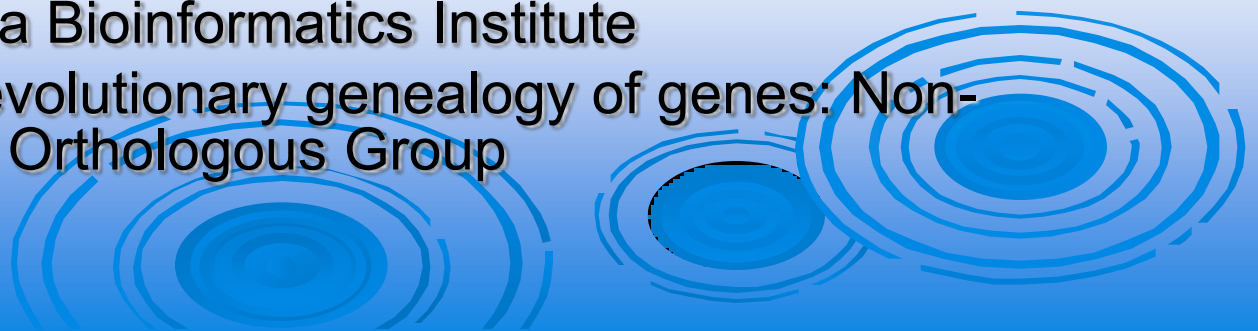
Functional metagenomic profiles

- Microbialite metagenome(s) sequenced by 454 shotgun
- Not intended to close metagenomes but will provide valuable reference baseline
- Generated a comprehensive baseline of functional genes in Shark Bay microbialites
- Submitted to Genbank as metagenome projectS (SEED, MG-RAST)
- Overall community metabolisms, carbon sequestration, adaptive responses, HGT, EPS, quorum sensing, nutrient cycling, novel genes, viruses...?

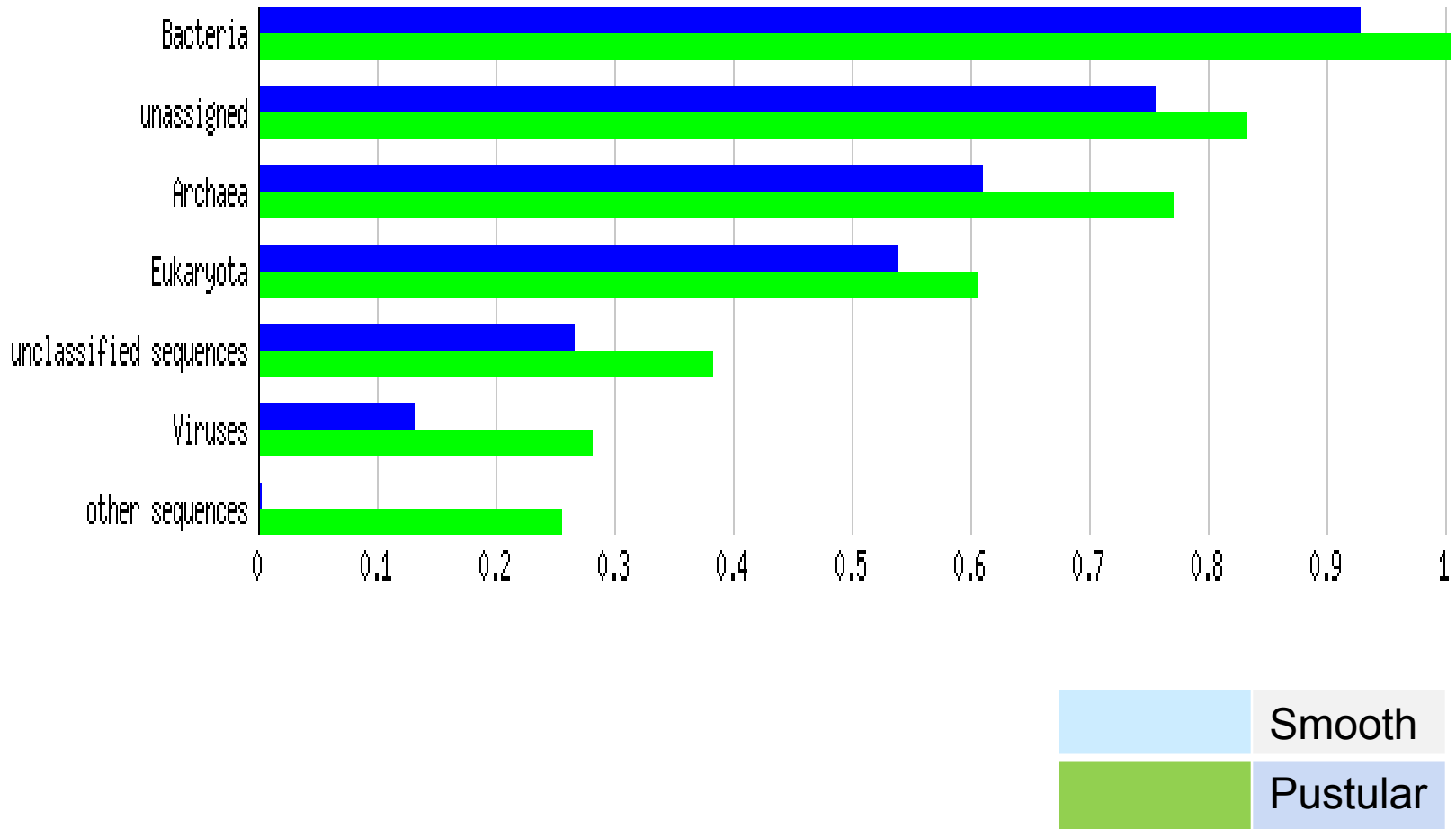


Annotation Source: M5NR- The M5 non-redundant protein database

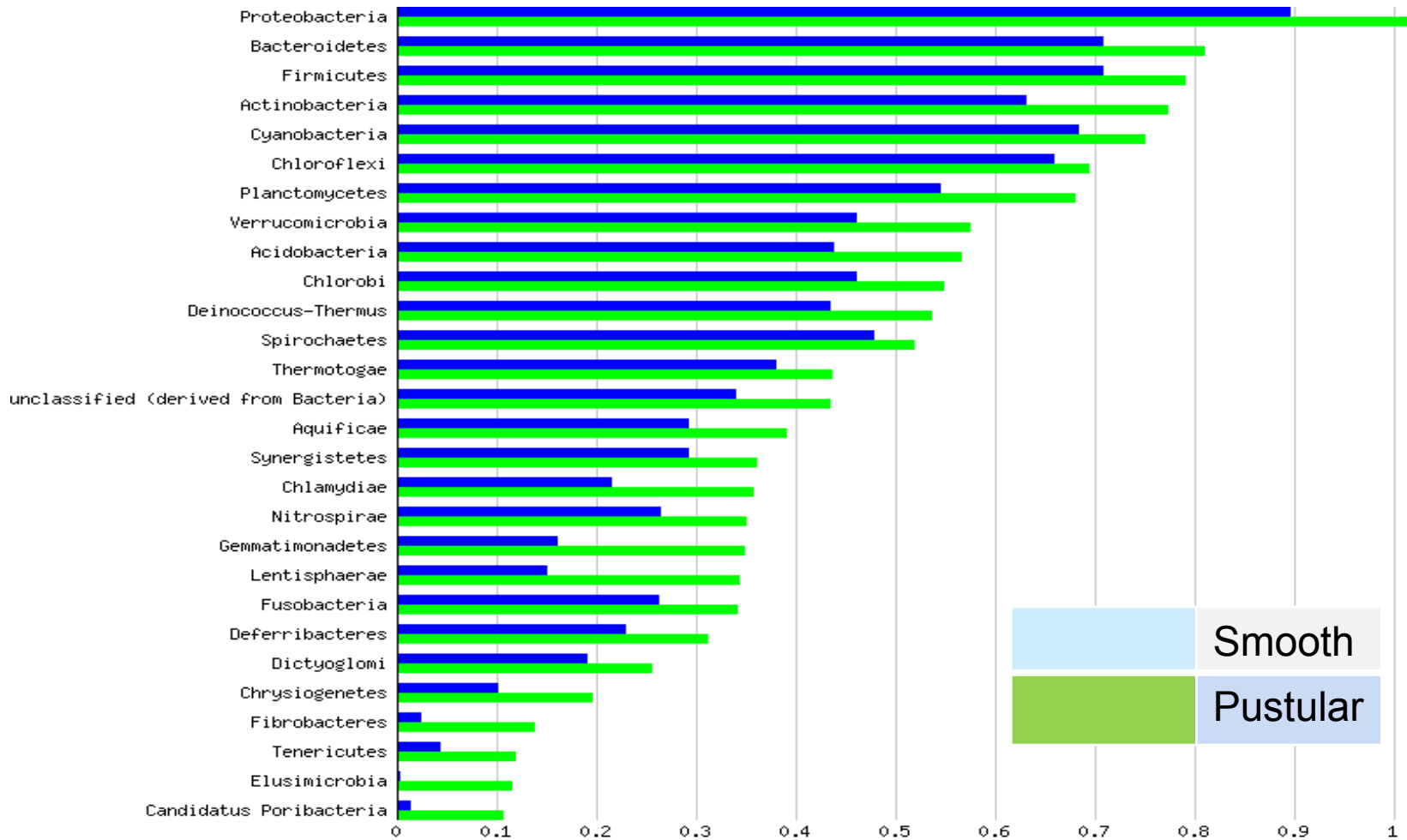
- The databases below are part of the M5NR
 - GO: Gene Ontology
 - Greengenes: 16S rRNA Gene Database
 - JGI: Joint Genome Institute
 - KEGG: Kyoto Encyclopedia of Genes and Genomes
 - NCBI: National Center for Biotechnology Information
 - RDP : Ribosomal Database Project
 - SEED: The SEED Project
 - SILVA: SILVA rRNA Database Project
 - UniProt: UniProt Knowledgebase
 - VBI: Virginia Bioinformatics Institute
 - eggNOG: evolutionary genealogy of genes: Non-supervised Orthologous Group



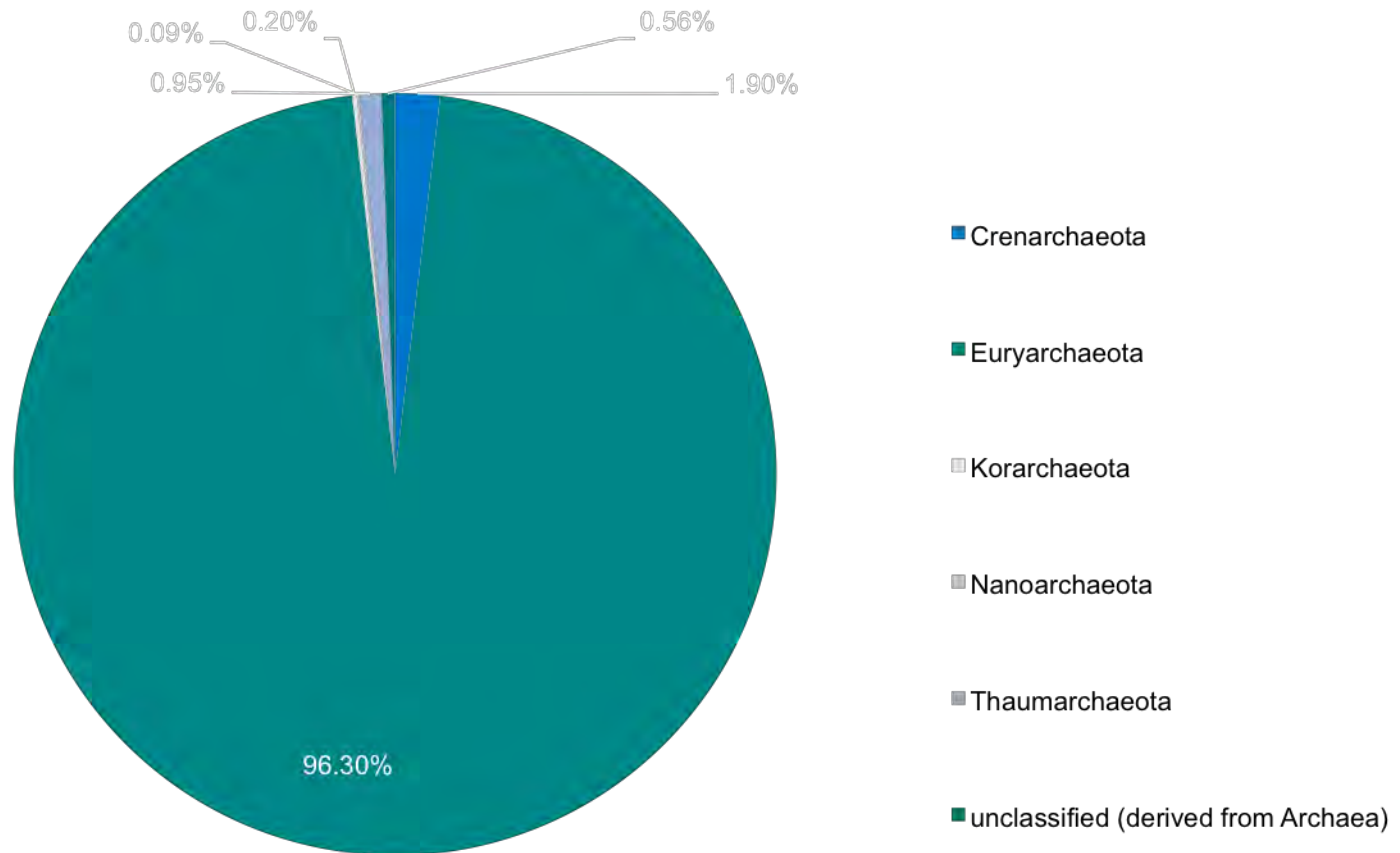
Microbial Diversity of Shark Bay microbialites - shotgun



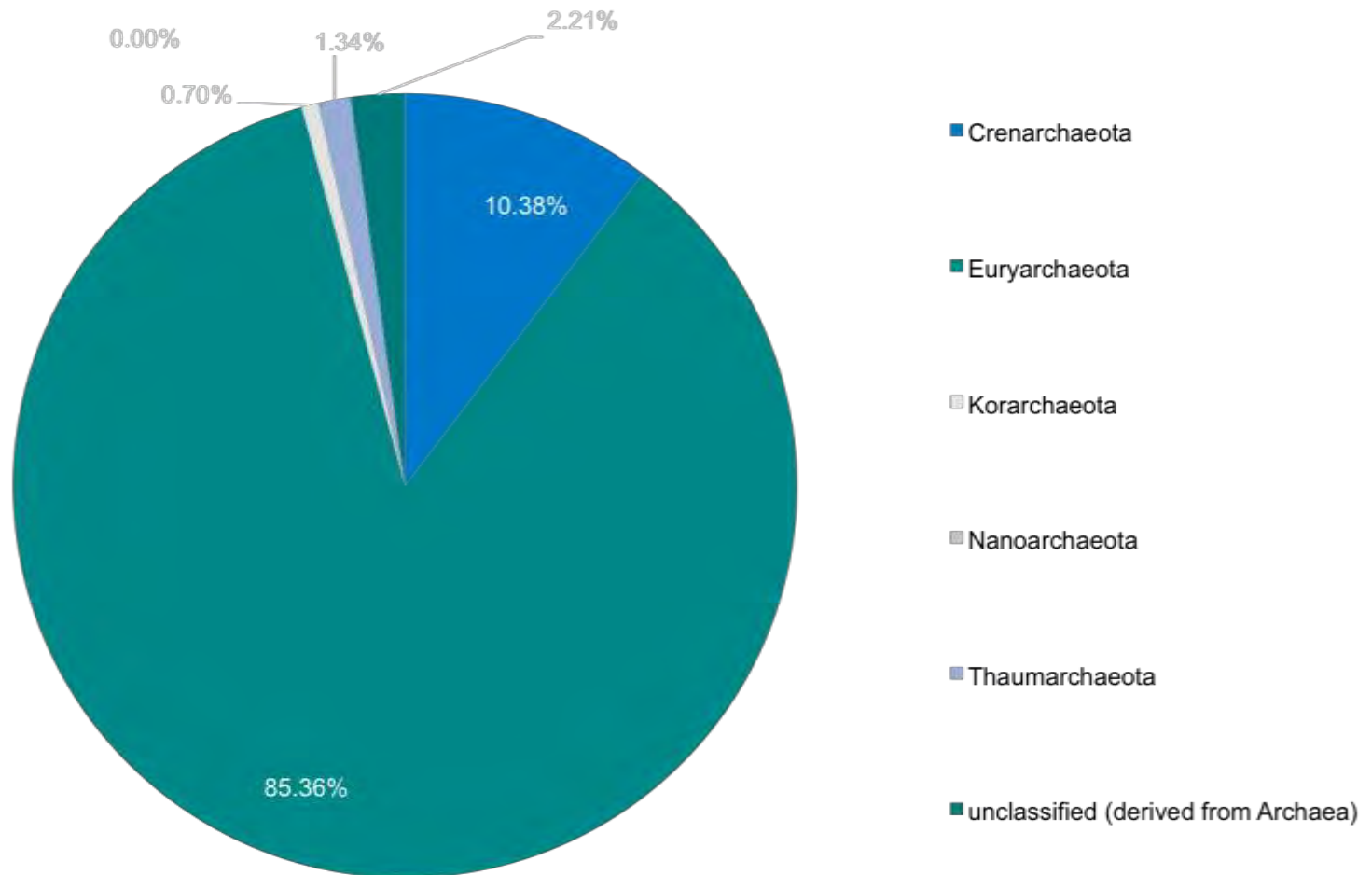
Bacterial Diversity (Phylum)



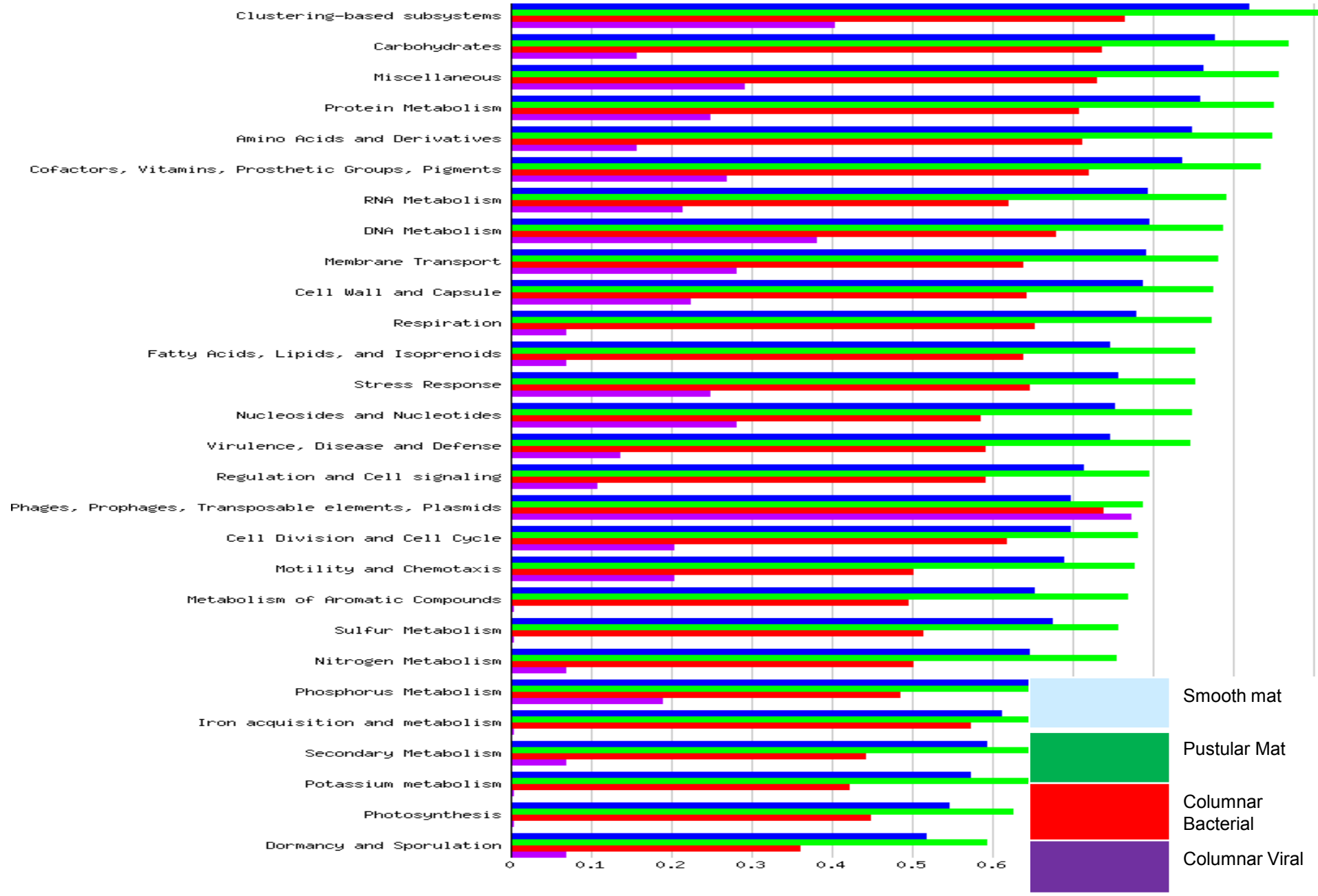
Archaeal Diversity in Pustular Mat (Phylum)



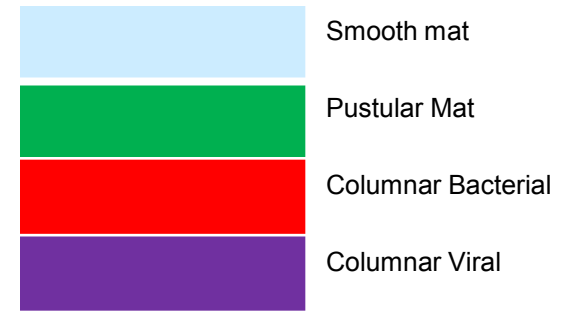
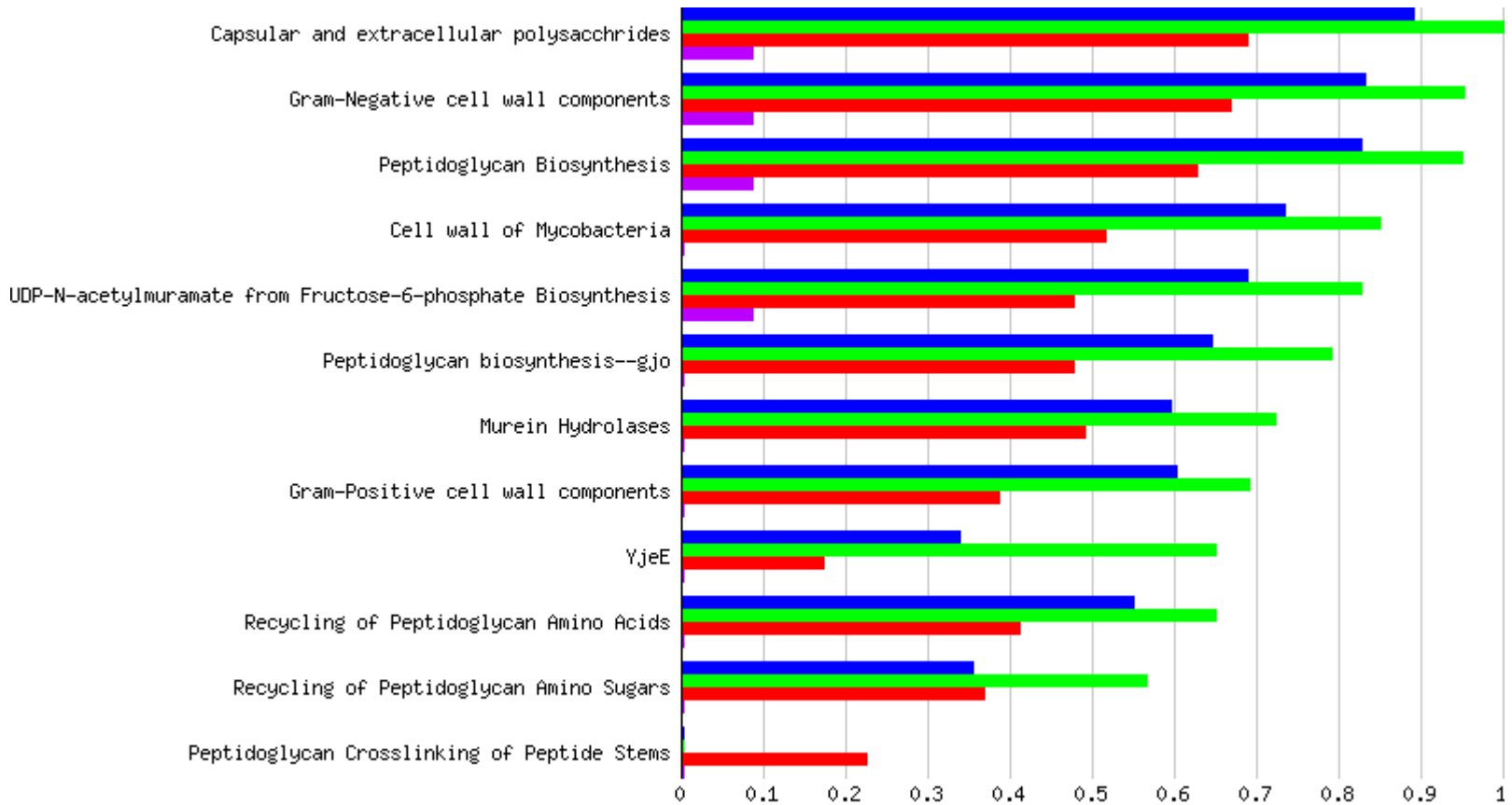
Archaeal Diversity in Smooth Mat (Phylum)



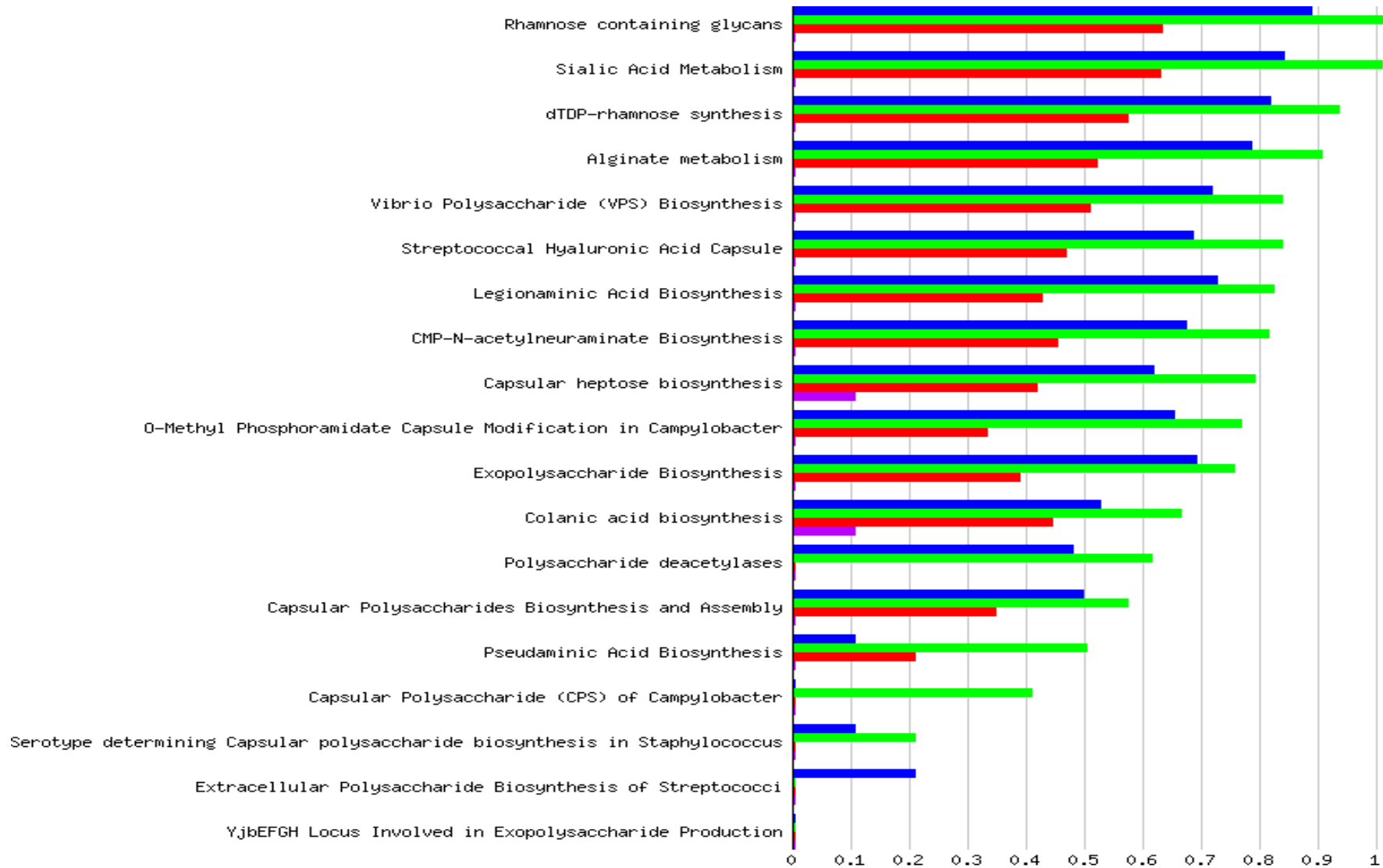
Functional Diversity of Shark Bay microbiliates



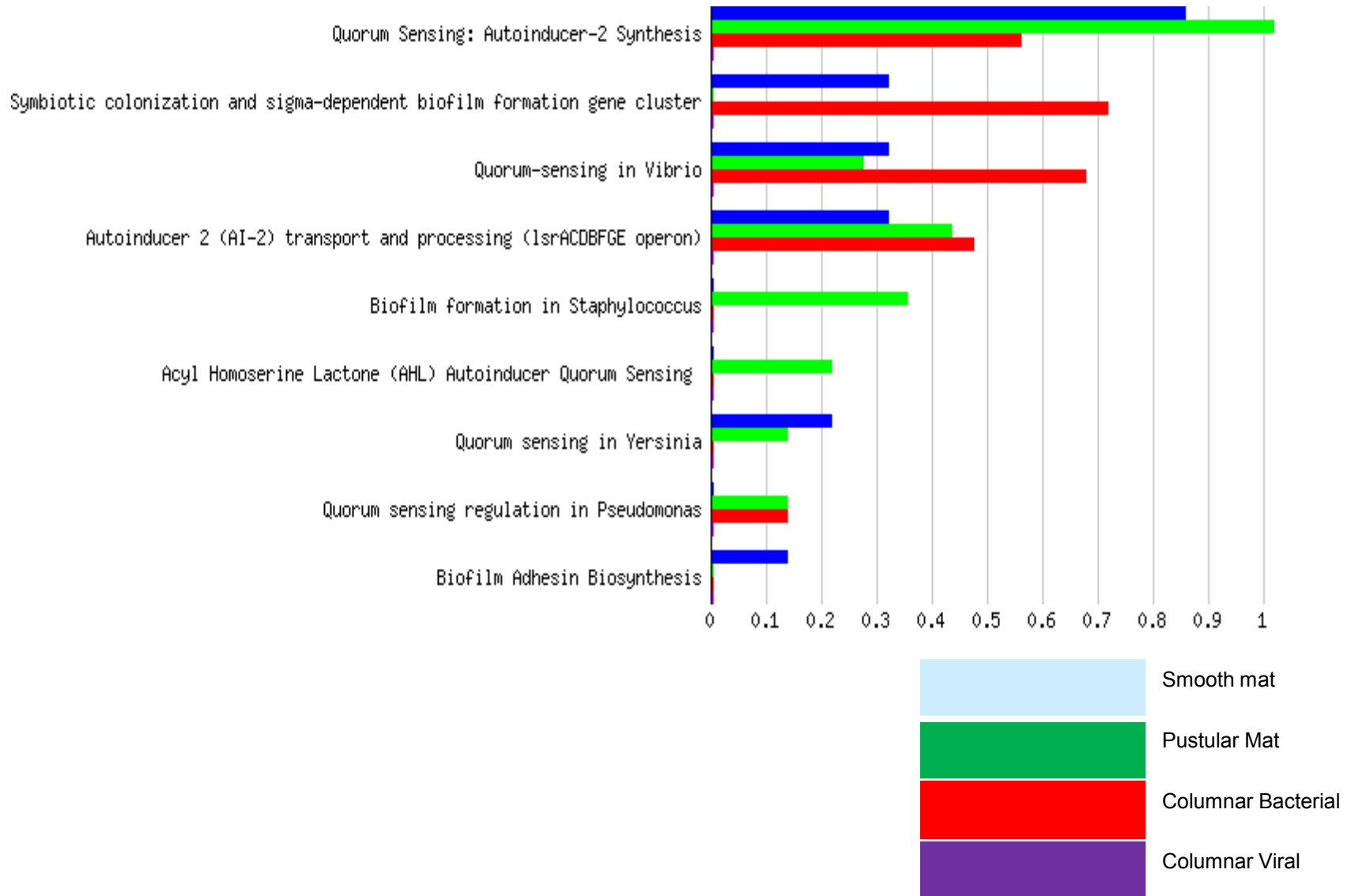
Capsular and Cell Wall



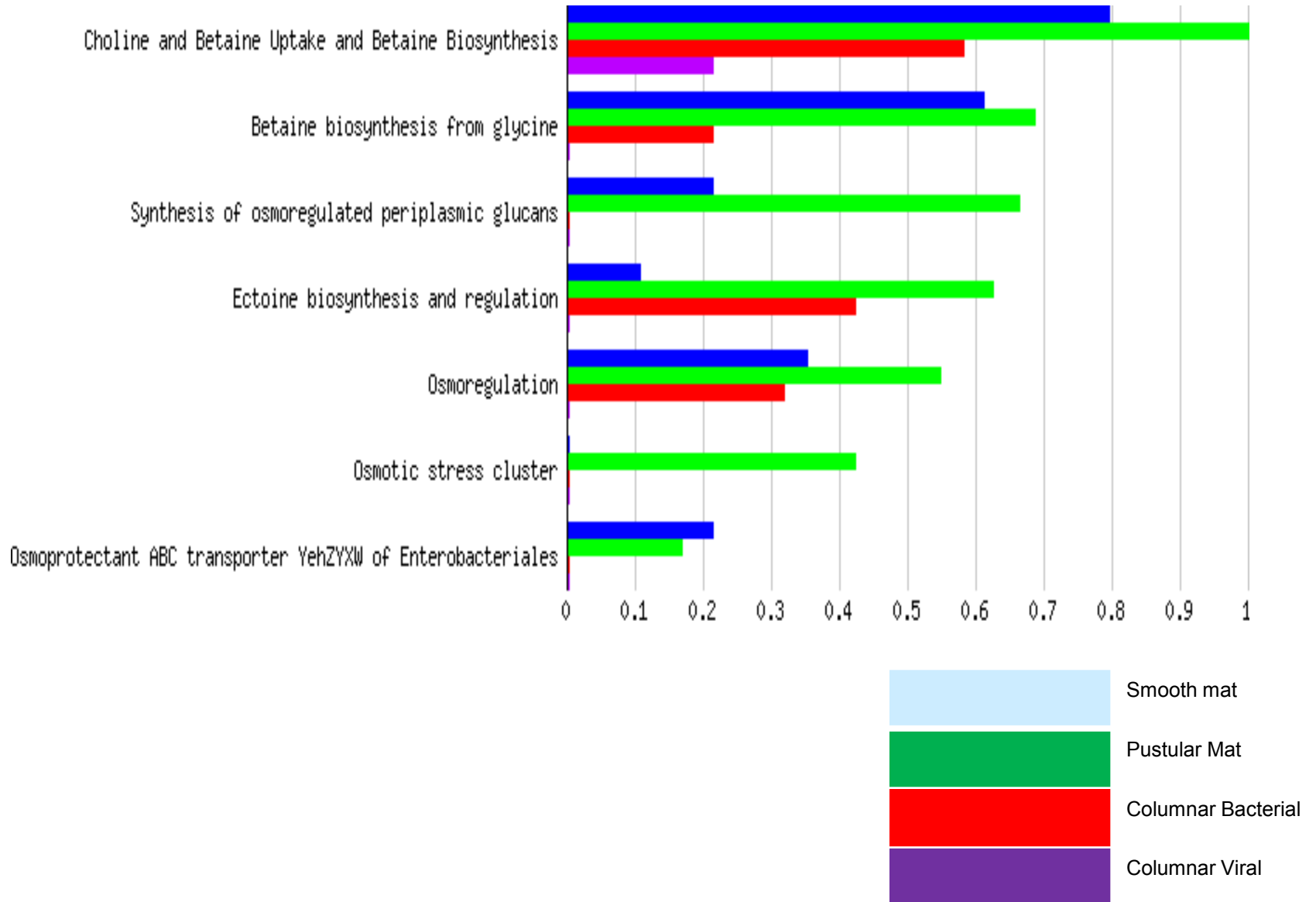
Capsular and EPS



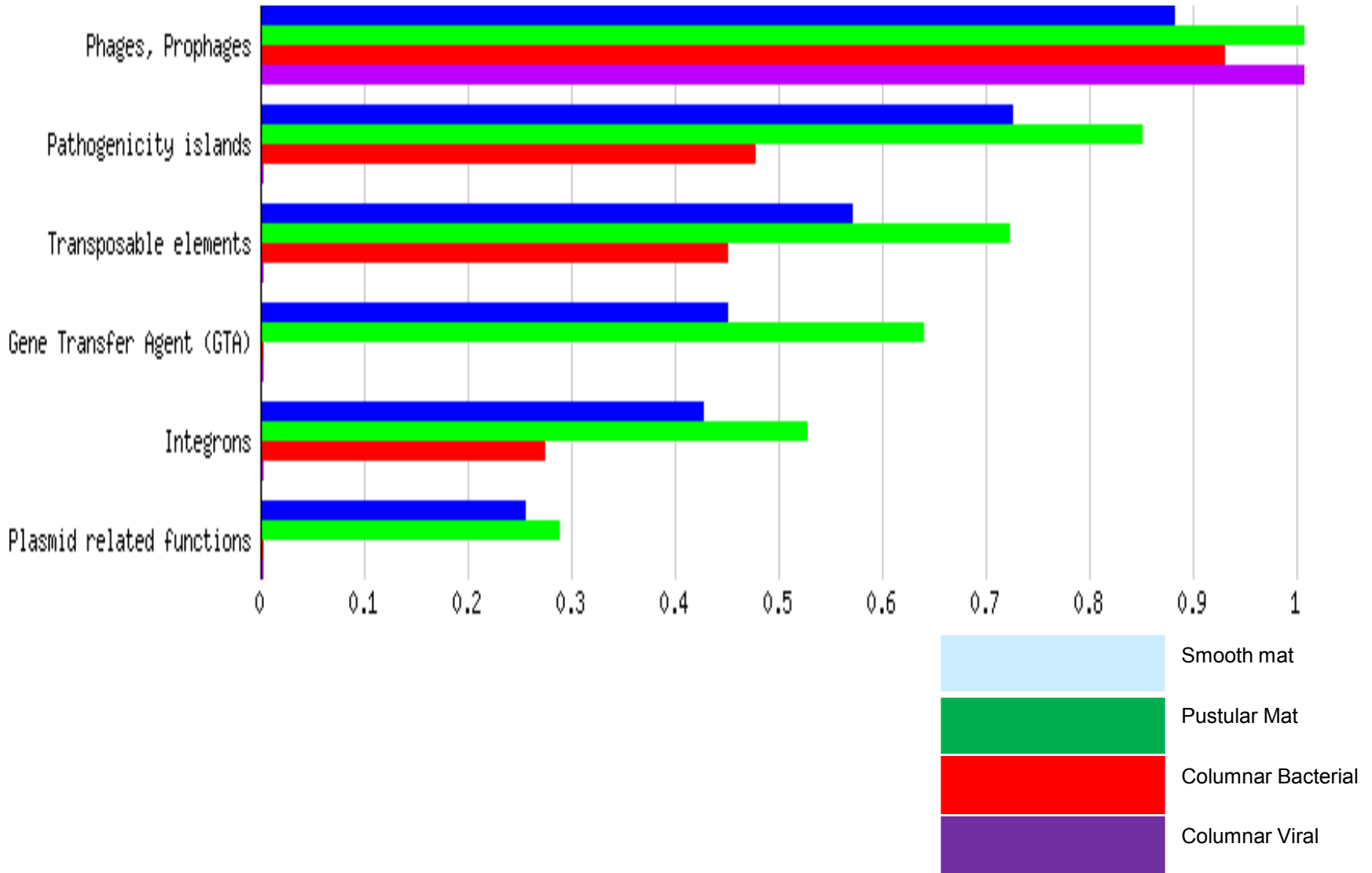
Cell Signalling



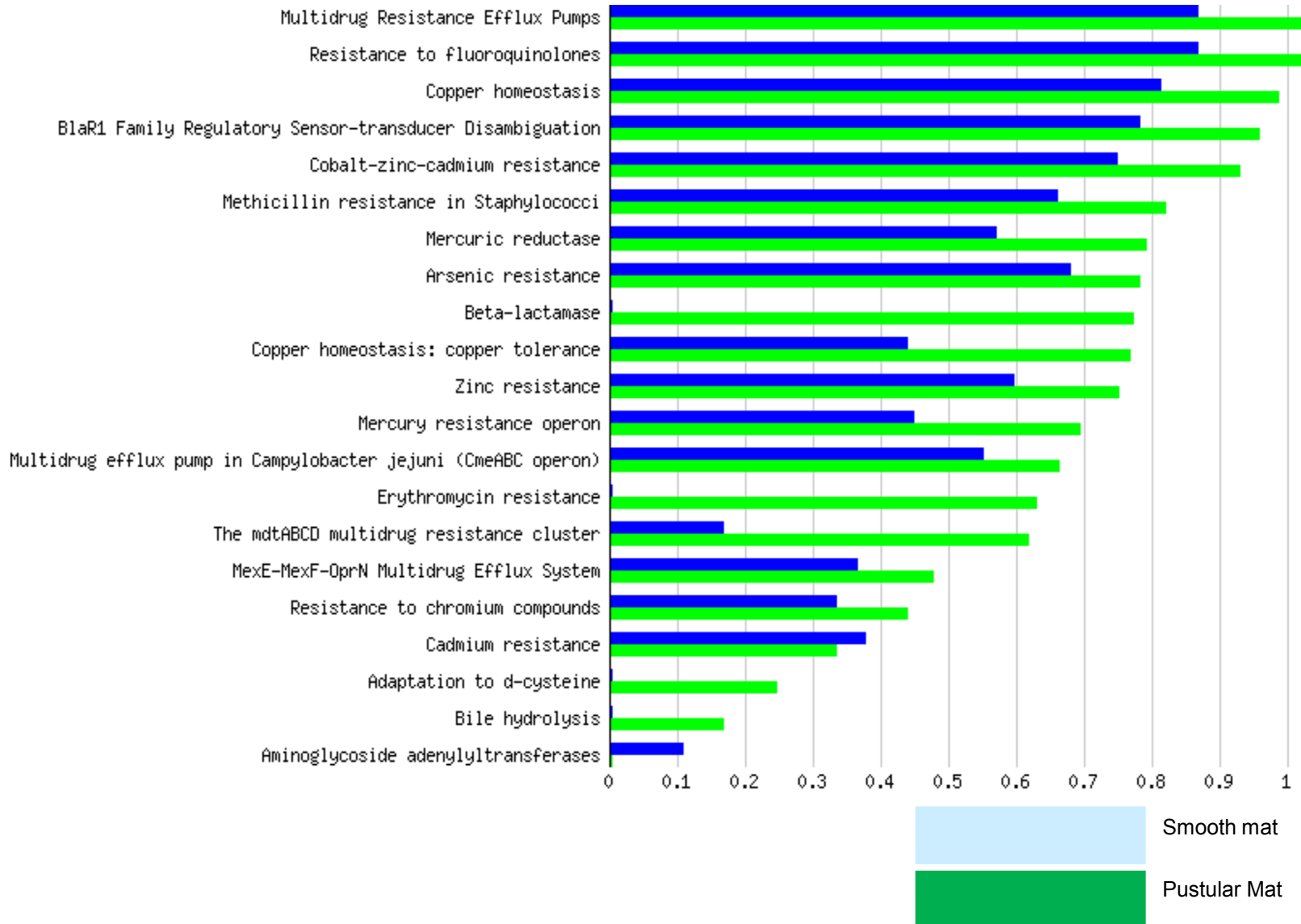
Osmoadaptation



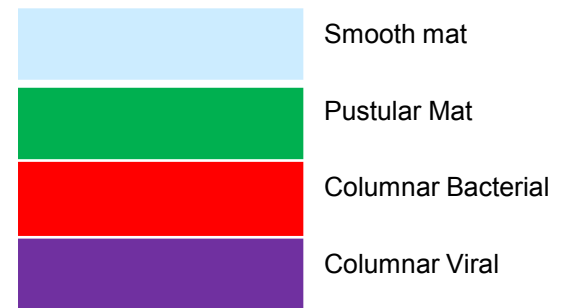
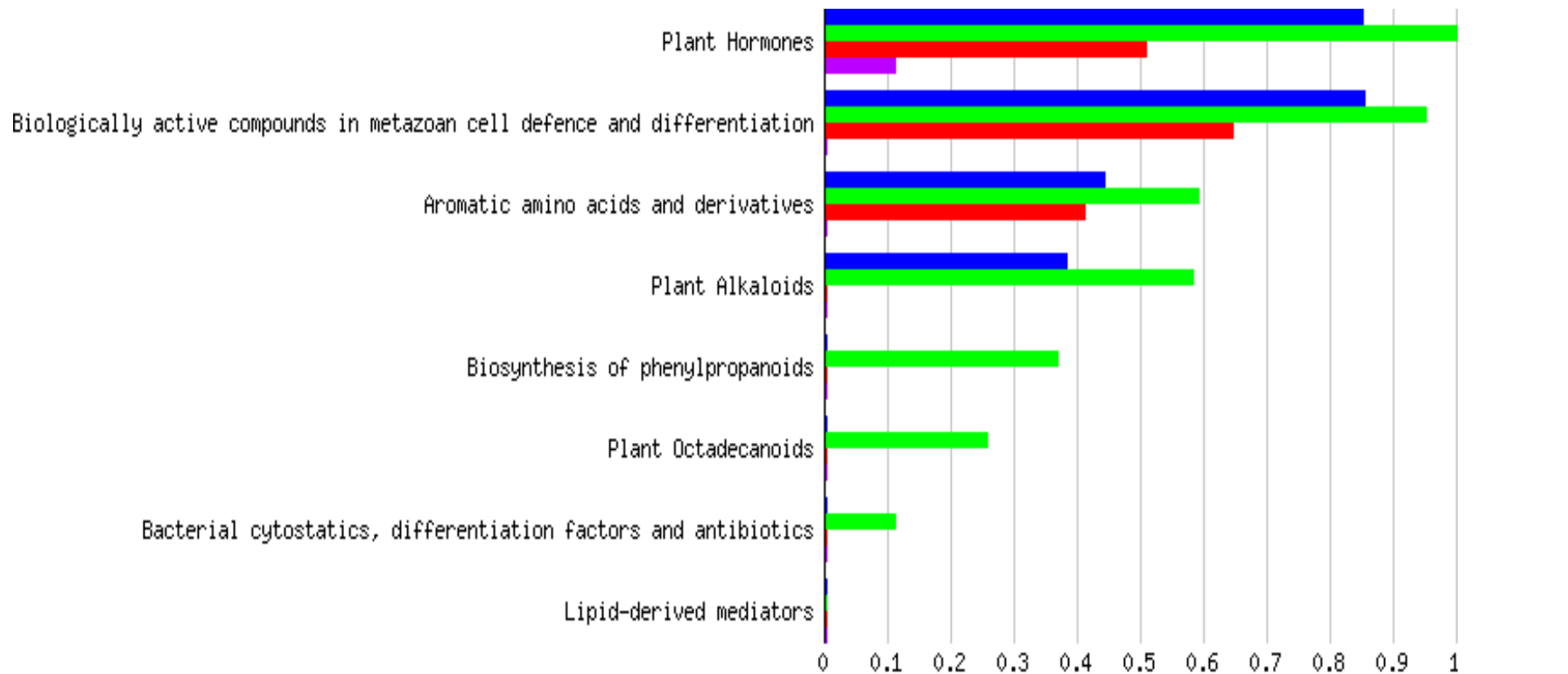
Horizontal gene transfer



Metal and Antibiotic Resistance

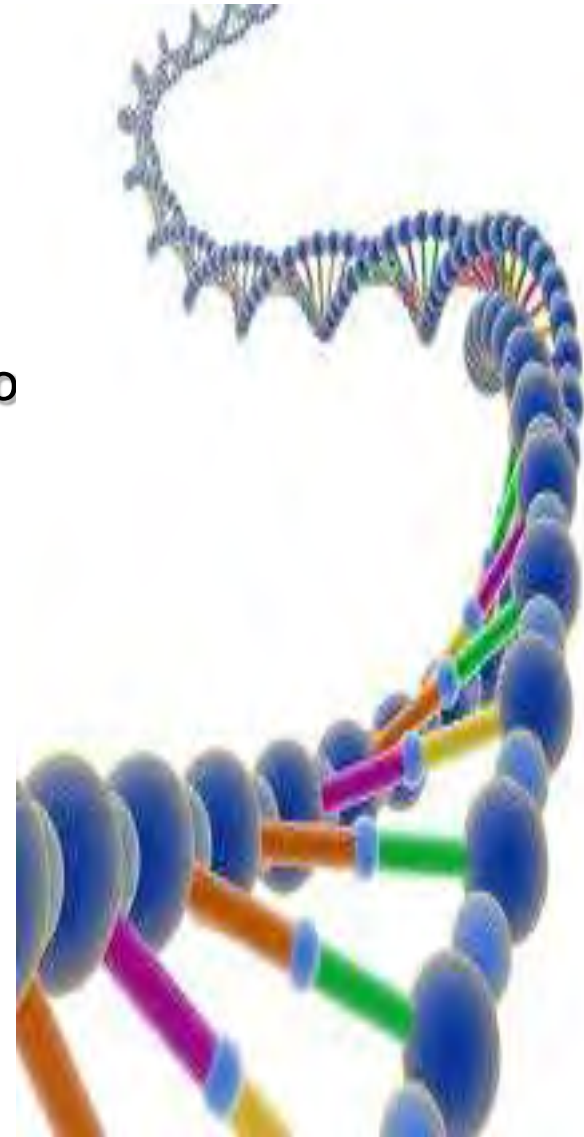


Bioactive compounds



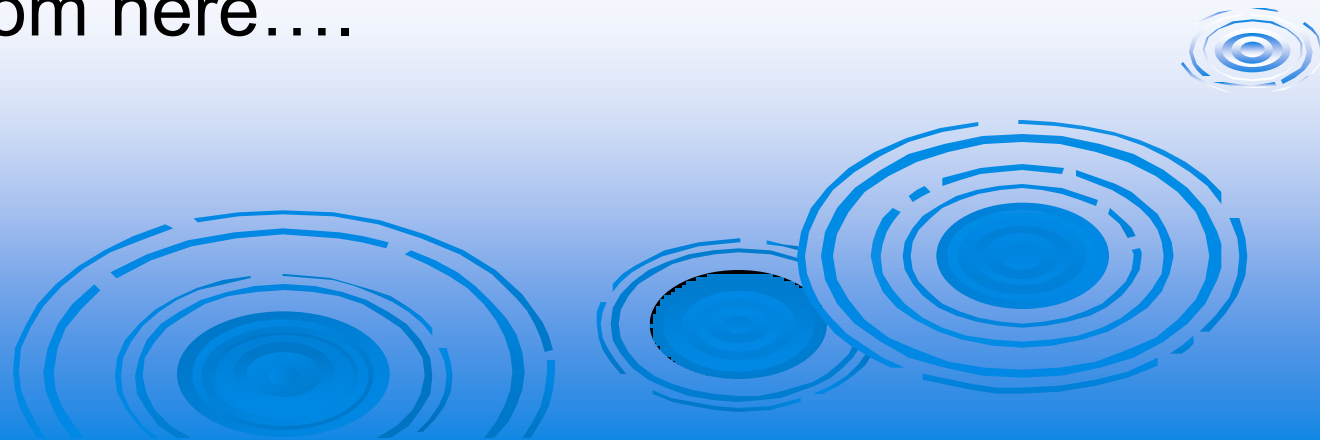
What about all those pesky unknowns....?

- ~ 30% of the metagenome unknown/hypotheticals
- These may represent some novel genes/pathways
- Deeper sequencing may result in re-annotation of so of these
- Conduct transcriptional analyses, look for conserved regions/motifs, employ filters (similarity/feature/pattern/binding finders) to help elucidate function of others



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Metabolic and genomic profiling of Shark Bay microbialites

- Aim is to perform biogeochemical, geological, chemical biological, and metagenomic analyses in such a way to obtain data on exactly the same samples
- Conduct *in situ/ex situ* field measurements followed by complementary lab experiments
- Focus on metabolisms intrinsic to strom formation/preservation and ecosystem function
- EPS turnover, Ca precipitation, cell communication, adaptation, HGT



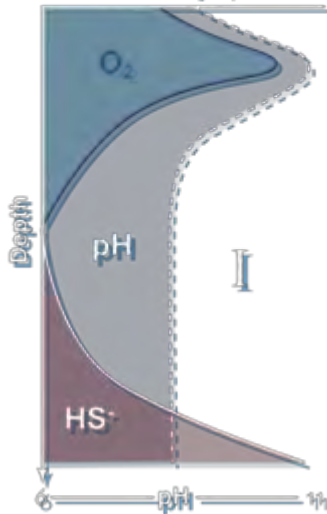
Major metabolic pathways in microbialites – nutrient cycling impacting microbialite formation



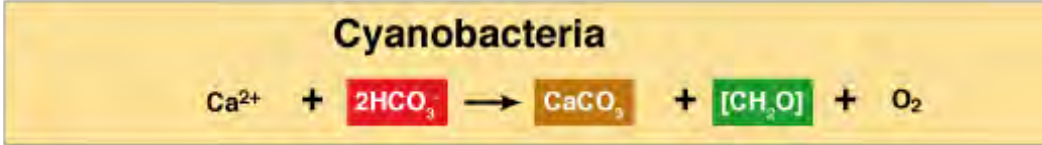
Promoting precipitation



Oxygen (µM) = 1500
Sulfide (µM) = 800

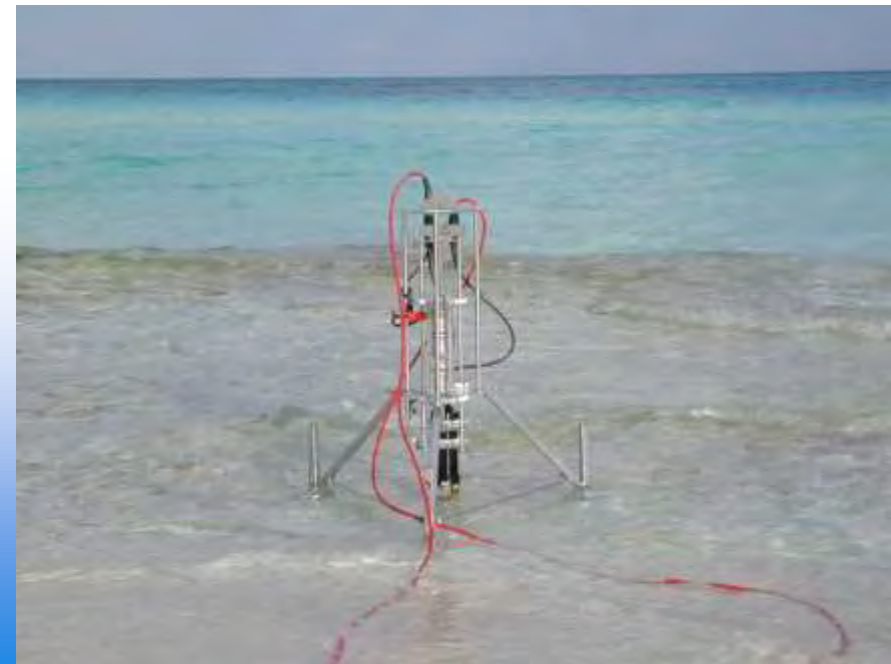


Promoting dissolution



Microelectrode profiling

- Measure changes in O and S turnover every 0.2 mm depth over diel cycle (correlate with light intensity, ph, Ca^{2+})
- Photosynthesis, respiration, and sulphur metabolism
- Show productivity (day/night), relate to specific organismal groups and spatial location within stromatolite







4x6
PACKS
(24 BOTTLES)

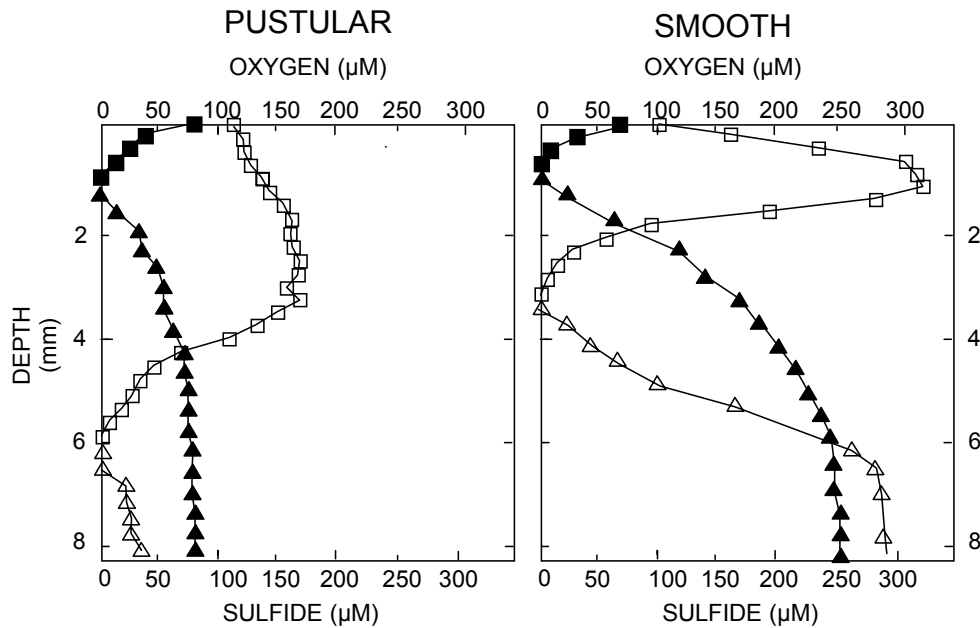
Coopers

ORIGINAL PALE ALE

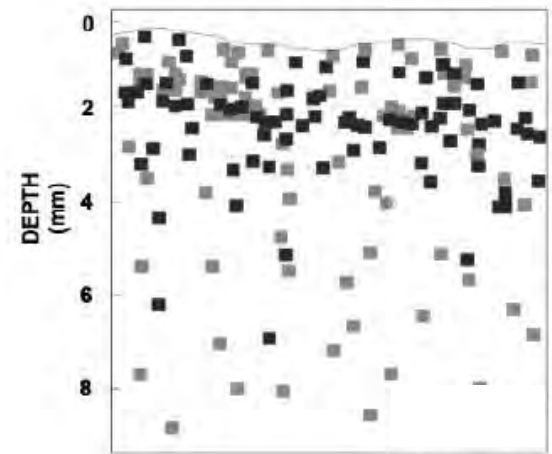
www.coopers.com.au



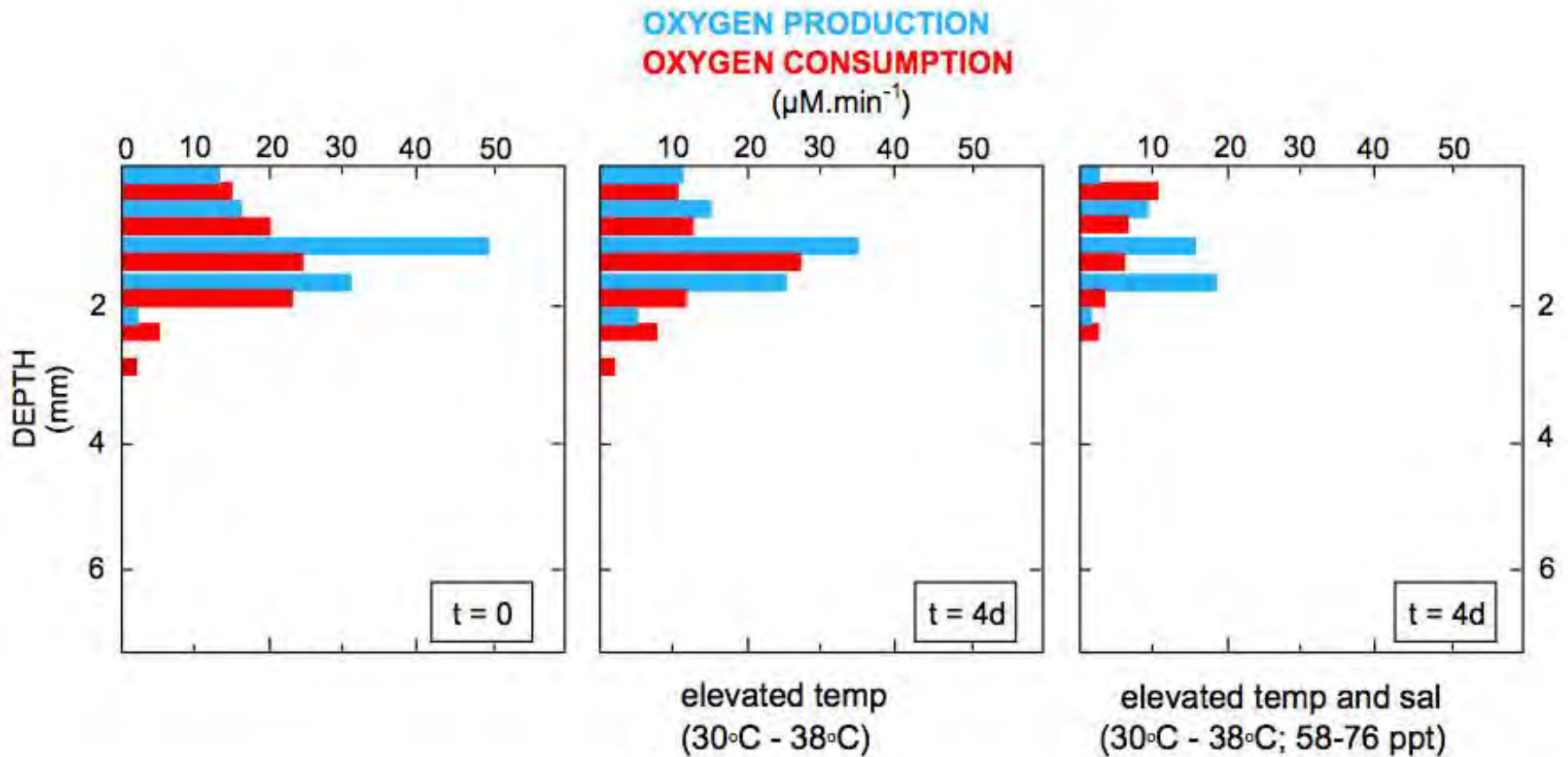
Geochemical gradients and microbial rates in Shark Bay microbialites



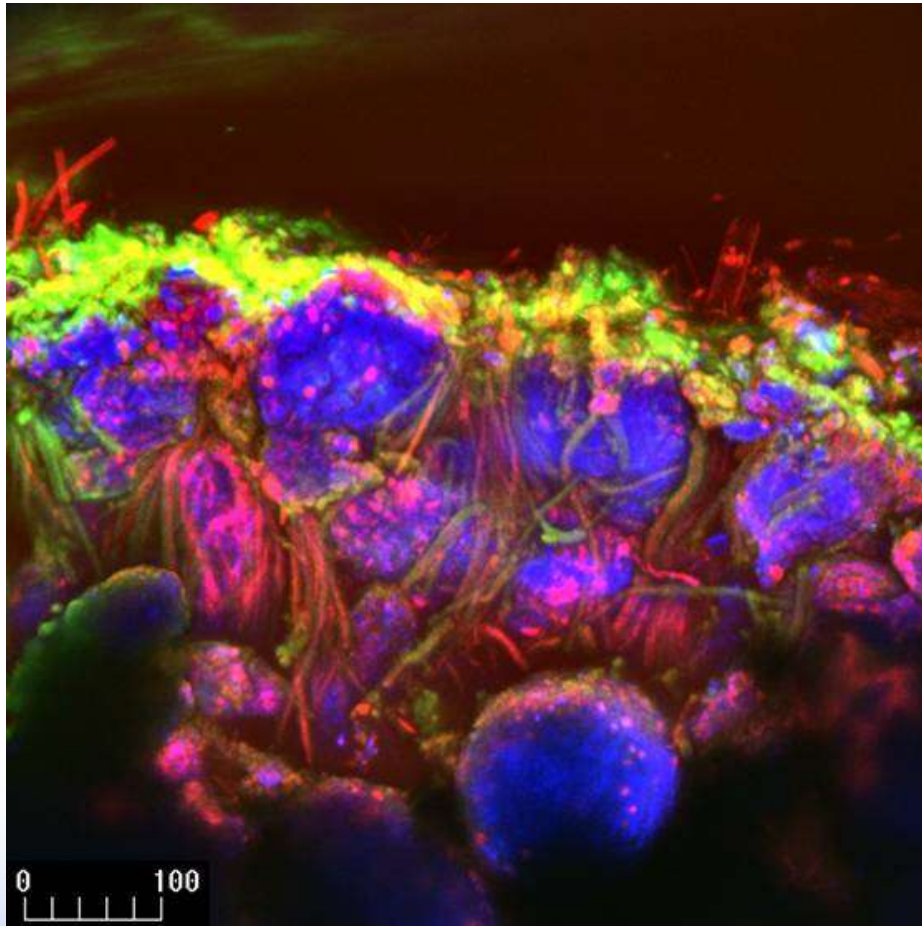
Sulphate reduction



Stress/climate change effects on ecosystem health



Physical organisation of stromatolite microbes

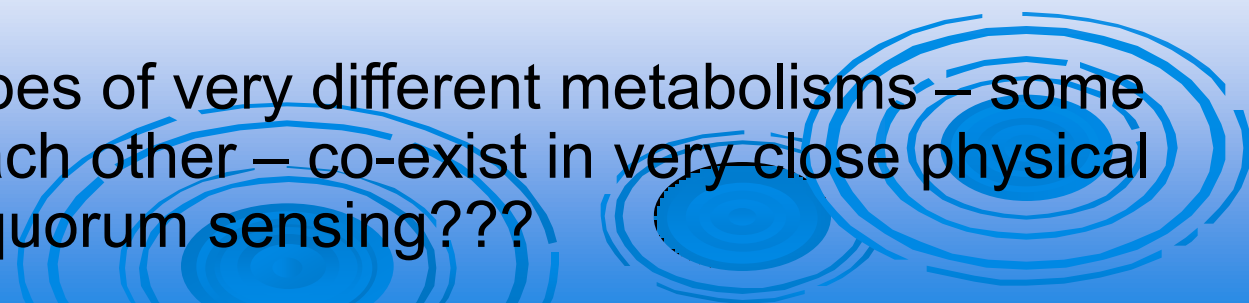


Green (FISH Probe): *dsrAB* (16S)
Blue (CaCO₃ Precipitate and Ooids)
Red Autofluor.(Cyanobacteria chl)

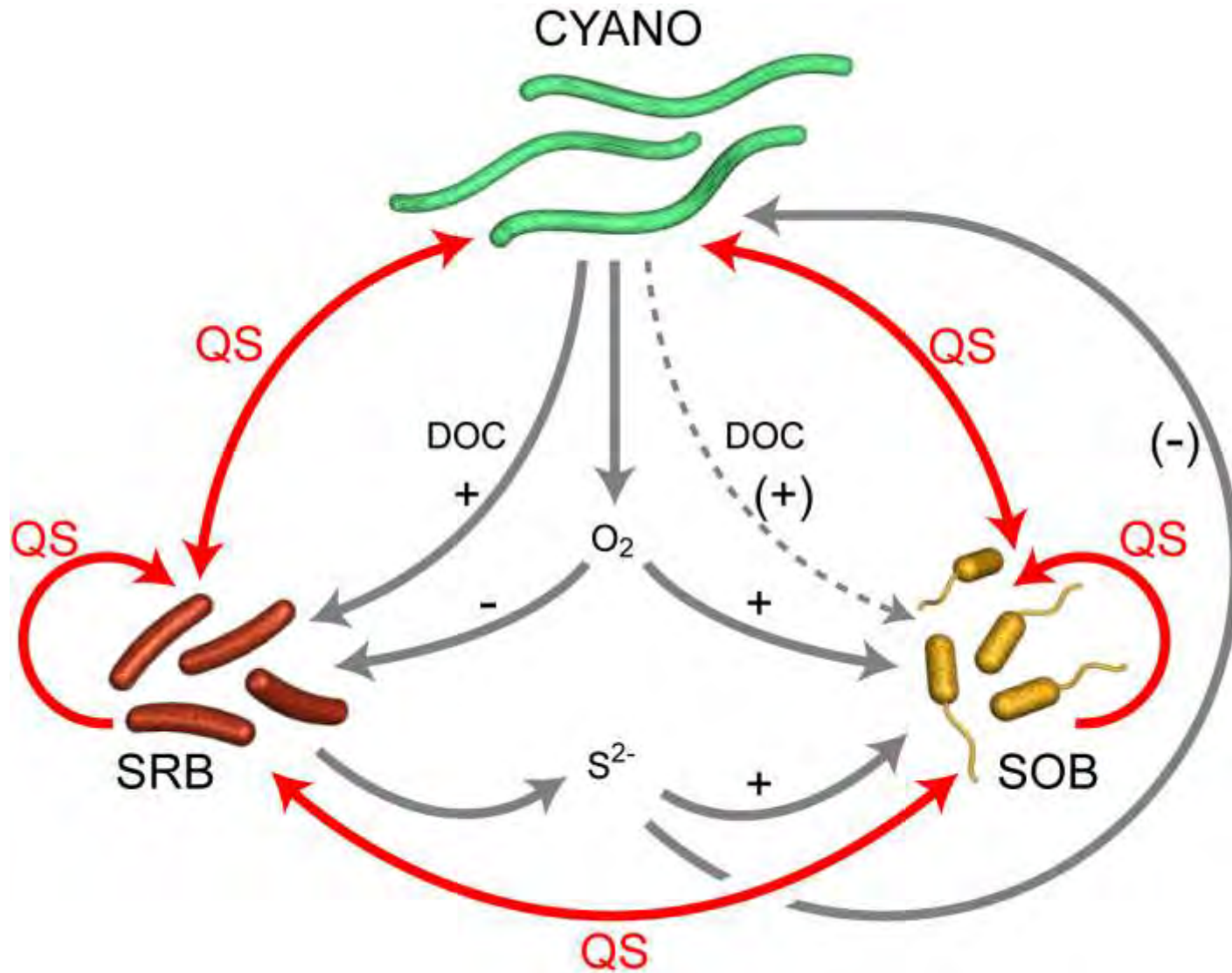
Decho and Visscher



How do microbes of very different metabolisms – some inhibitory to each other – co-exist in very close physical proximity.....quorum sensing???

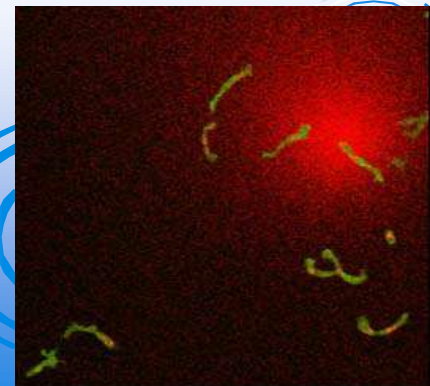


Metabolic cooperation?



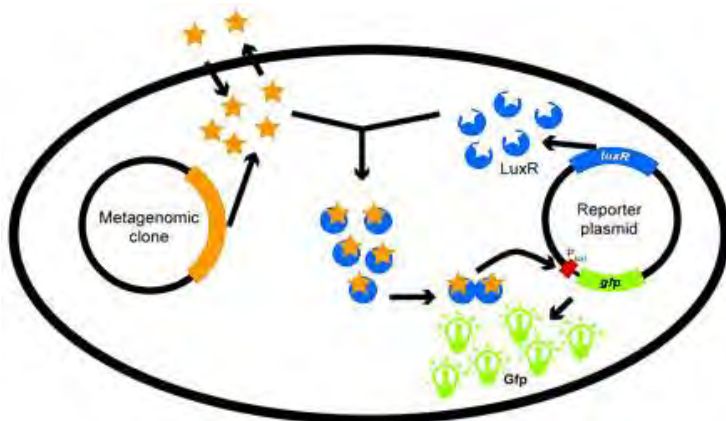
Metabolic cooperation in microbialites

- Signaling might occur between different functional organismal groups to facilitate (and periodically adjust) efficient recycling of nutrients in microbial mats
- This synergy could allow both SRB and SOB to thrive under seemingly unfavorable environmental conditions (high O_2 , and lack of sulfide, respectively)
- Signaling may help balance very different metabolisms in a microbial community

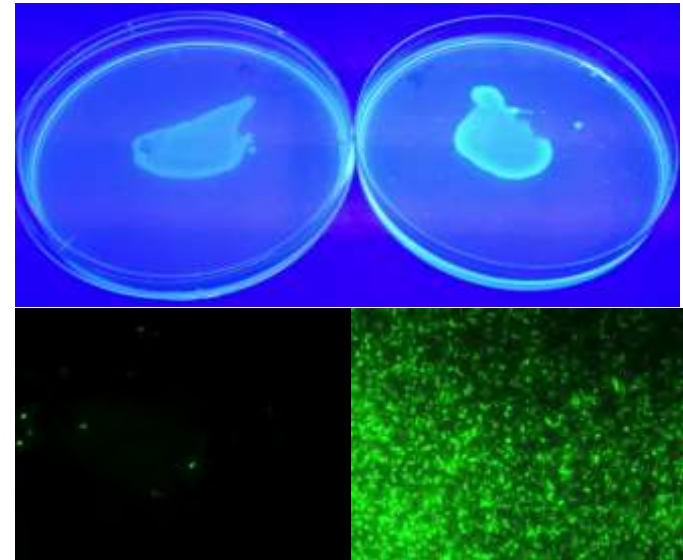


Signalling in Shark Bay microbialites

- Recent work demonstrated quorum sensing in Bahaman mats (Decho, Visscher et al 2009, 2010)
- Likely to be intrinsic to ecosystem function – regulate gene expression, phenotypes
- Tested extracts from Shark Bay microbialites for signals using range of QS biosensors and mass spectroscopy – success!
- Archaea have joined the conversation...



Williamson et al (AEM 2005 71, 6355)



Outcomes of this research program

- Link key metabolisms and their related genes to ecosystem function and stromatolite formation/preservation
- Flow of key metabolites in these microbial systems – creation of 3-D reconstruction of key metabolic activities
- Relate specific activities to mineral products and resulting biosignatures that can be detected in the fossil record
- Uncover genetic traits specific to these ecosystems
- Ultimately develop a virtual field trip once all data is obtained – Gigapan technology



Limitations

- Extracting DNA from environmental samples notoriously tricky
- Any bias needs to be reduced
- How deep to sequence, where do you stop?
- Important not to sequence for sequencing sake, need to have rational, focused questions to address
- Often providing a snapshot but is it representative over a long time-frame?
- Data sets becoming HUGE....need for excellent computational/bioinformatics support

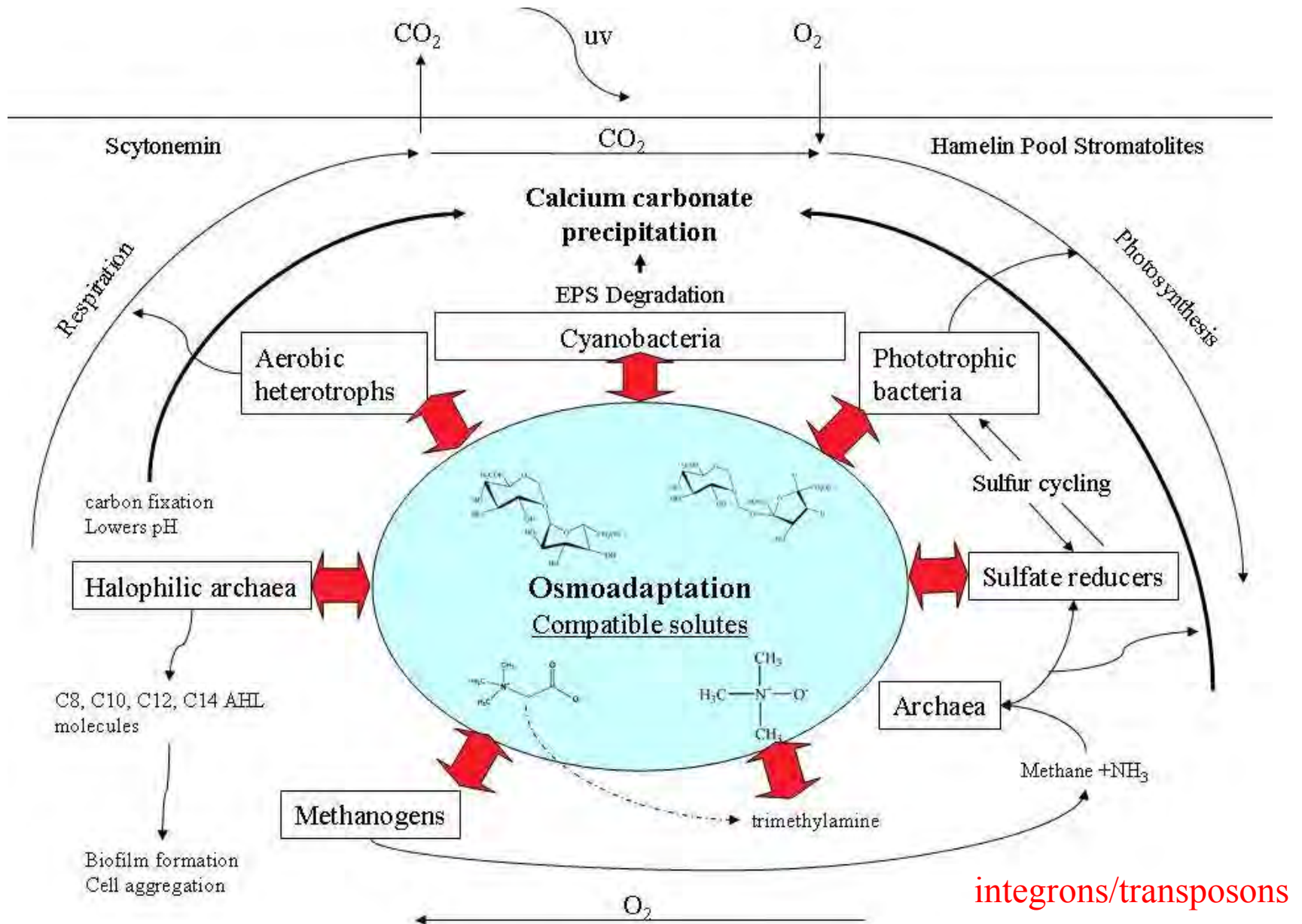


Summary of focal research points

- Shark Bay microbialites possess incredible biological diversity which is reflected in their functional complexity
- Novel organisms/interactions elucidated - archaea
- Microbial metabolic processes alter the geochemical environment – key metabolisms (photosynthesis, respiration, S reduction) tightly coupled
- Next generation sequencing unraveled incredible genetic diversity and potential of microbialite ecosystems
- Intrinsic value of focused metabolic/physiological studies (eg. microelectrode profiling, EPS characterisation, culture studies) in addition to genomic-based

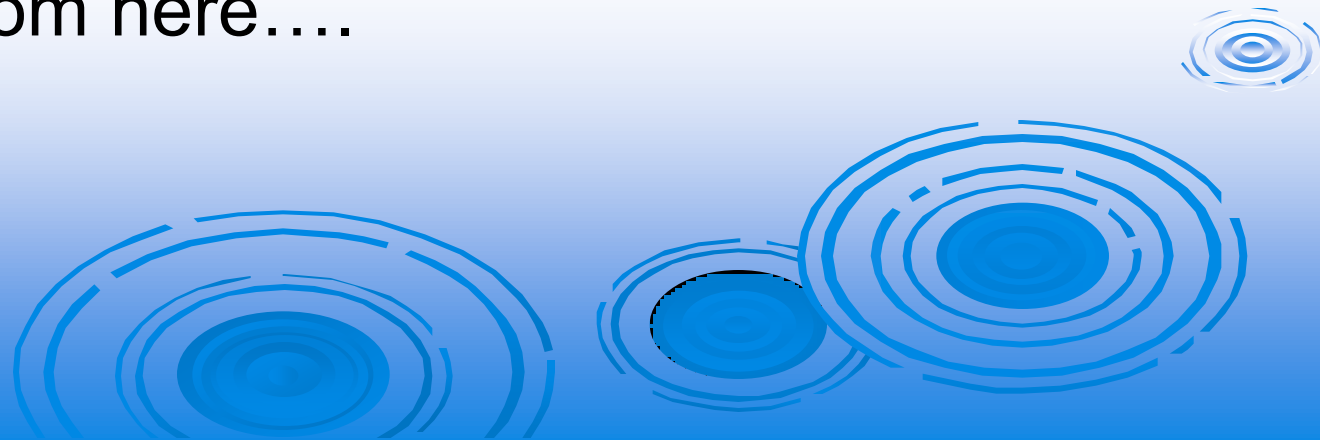


Hypothetical model



Outline

1. Shark Bay microbialites
2. Initial diversity studies
3. Tagged pyrosequencing
4. Functional metagenomic sequencing
5. Microelectrode profiling
6. **Conservation and sustainability**
7. Where to from here....



Garnaut Climate Change Review

Impacts of climate change on Australia's World Heritage properties and their values

Prepared by

Lance Heath, ANU Institute for Environment, The Australian National University

June 2008

“.....Australia's World Heritage property at Shark Bay contain „outstanding examples representing major stages of earth's history, including the record of life, significant on-going geological processes in the development of landforms, or significant geomorphic or physiographic features....’

.....”Shark Bay will face a more immediate threat from further sea level rise causing an increase in flooding of low lying areas.”

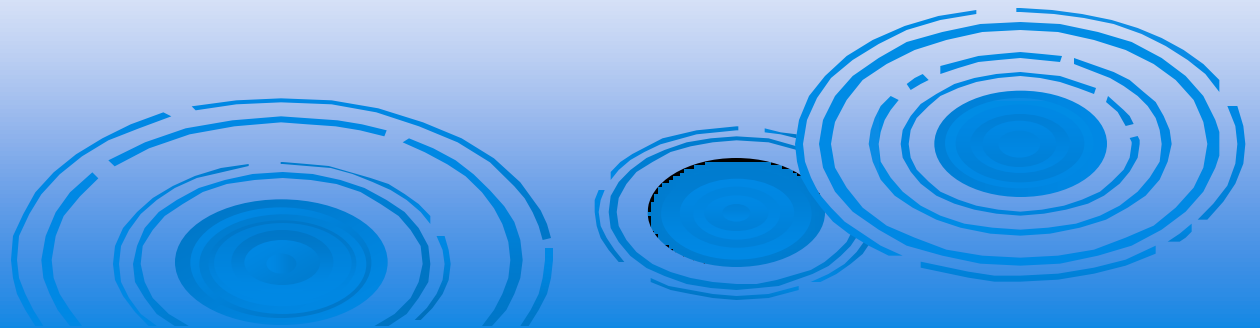
Biodiversity and sustainability

- Conservation of stromatolite biodiversity further drive of this research
- Microbialites very vulnerable to human impact
- Temporal reduction in microbial diversity can indicate detrimental impact
- Evidence already of increased phosphate due to agricultural activities
- Microbialites are excellent markers to the health of our planet
- Need to effectively manage earth's ecosystems in the face of environmental challenges (global warming, pollution, climate change...)



Outline

1. Shark Bay microbialites
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Ongoing/future research directions

- Metatranscriptomics (eg, day/night, salt, different strom types)
- Stress responses - real time PCR, knockouts, iTRAQ, osmolyte synthesis
- Sensing in mat systems (quorum sensing, osmosensing, chemosensing)
- Effect of environmental parameters (eg, salinity, UV) on community metabolisms - photosynthesis, N₂ fixation, mineral precipitation
- EPS, CaCo₃ precipitation and salinity
- Gene transfer - transposases, integrons, viruses?
- Microbe-microbe interactions (unknown microbes)



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