Ecosystem biomonitoring with eDNA: Metabarcoding across the tree of life in a tropical marine environment

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eDNA Analysis Methods

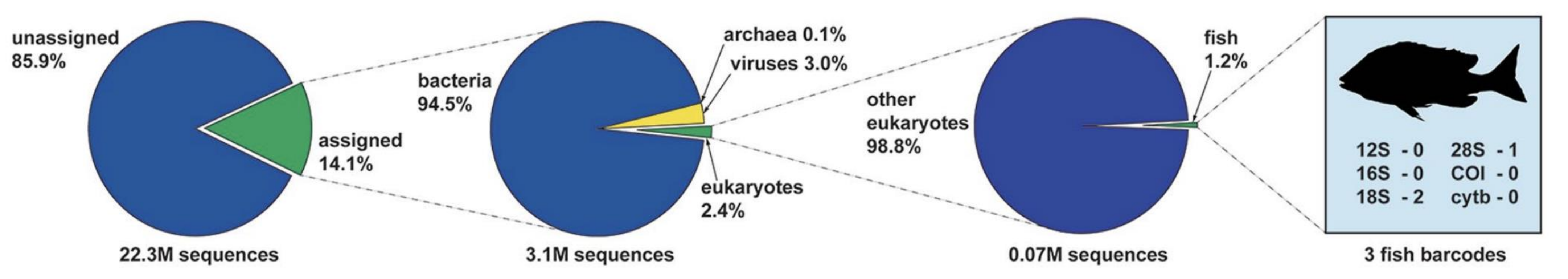
- Environmental Shotgun Sequencing (ESS)
  - Randomly sequences fragmented DNA directly from environmental sample
  - Cost prohibitive
  - Hasn’t been widely used for eukaryotes
- DNA Metabarcoding
  - PCR Amplification of target genes on bulk DNA extracts + next-generation sequencing (NGS)
  - Potential PCR bias

eDNA Method Survey

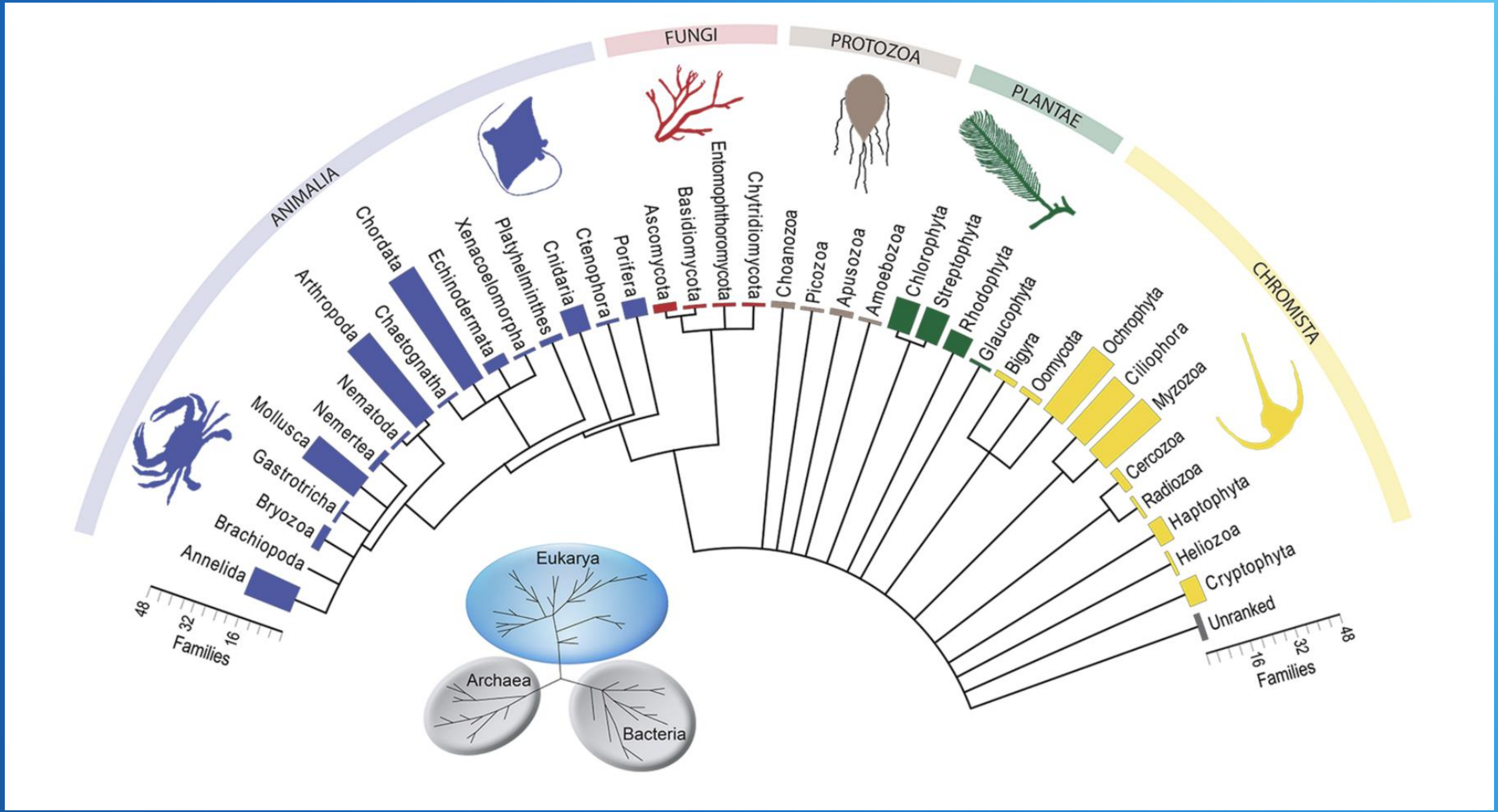
- Focused on single tropical reef site (Coral Bay in west Australia)
    - Renowned for marine biodiversity
- Explored utility of eDNA methodologies to assess eukaryotic diversity on ecosystem level
- Validated extent to which metabarcoding can inform on marine biodiversity
- Determined capacity for eDNA analysis from seawater

ESS from seawater

- Analyzed over 23 million sequences from 9L of filtered seawater

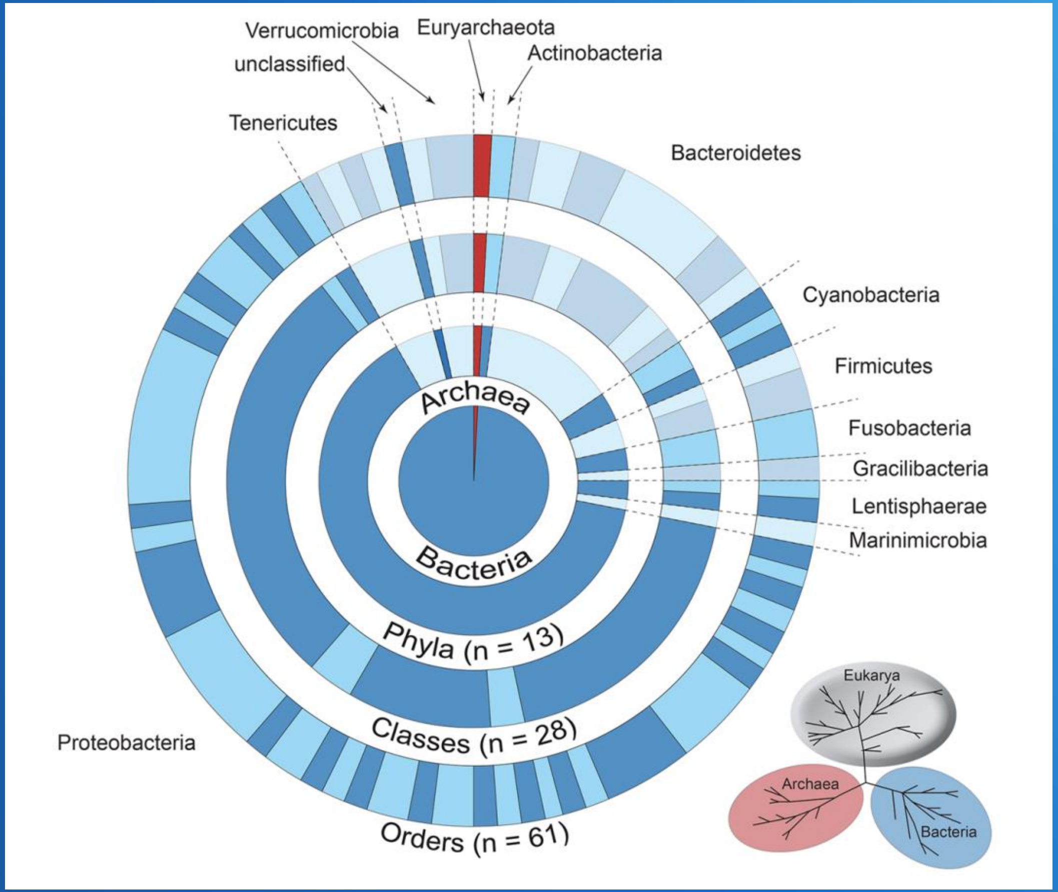


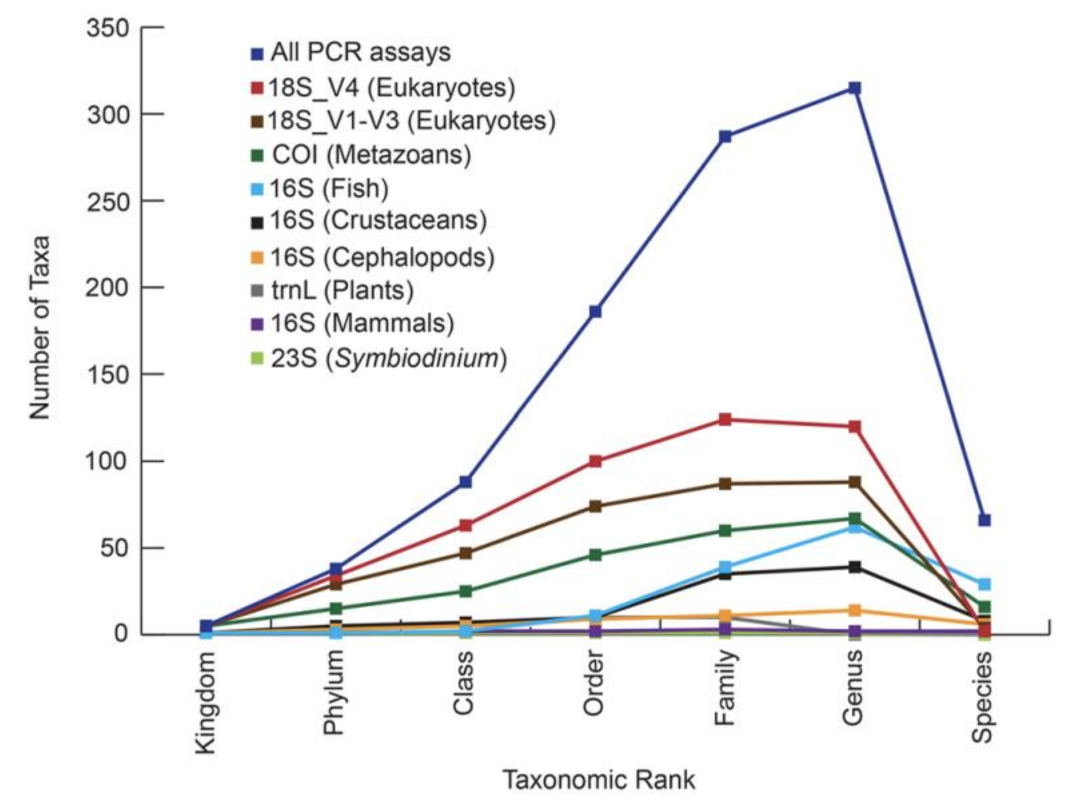
Tree of Life (ToL) Metabarcoding



Kingdom	Phylum	Class	Order	Family	Genus	Species
Animalia	Annelida	2	7	17	16	
	Arthropoda	3	12	35	38	8
	Brachiopoda	1				
	Bryozoa	1	1	3		
	Chaetognatha		1	1	1	
	Chordata	5	17	45	73	36
	Cnidaria	2	5	9	7	
	Ctenophore	1	1	1	1	
	Echinodermata	3	2	3	4	2
	Gastrotricha		1	1		
	Mollusca	4	15	22	19	5
	Nematoda	1	1	1		
	Nemertea	2	1	2	2	
	Platyhelminthes	4	3	2		
	Porifera	3	6	6	6	
	Xenacoelomorpha	1		1		
Chromista	Bigyra	1	2	2	3	
	Cercozoa	4	4	3	4	
	Ciliophora	7	15	24		
	Cryptophyta	3	3	6	6	
	Haptophyta	4	5	5	4	1
	Heliozoa	1	1	1	1	
	Myzozoa	3	10	25	36	2
	Ochrophyta	9	31	27		
	Oomycota	1	3	2		
	Radiozoa	2	2	1	1	
Fungi	Ascomycota	3	4	3		
	Basidiomycota	2	2	1	1	
	Chytridiomycota	1	1	1	1	
	Entomophthoromycota	1	1	1	1	
Plantae	Chlorophyta	7	10	11	17	
	Glaucophyta	1		1	1	
	Rhodophyta	1	5	7	3	
	Streptophyta	1	11	11	2	
Protozoa	Amoebozoa		1	1	1	
	Apusozoa			2	3	
	Choanozoa	2	1	2	3	
	Picozoa	1	1	1		
Total	**38**	**88**	**186**	**287**	**255**	**54**

ToL metabarcoding cont’d





ESS vs ToL metabarcoding

- ToL metabarcoding required less sequencing effort
- Greater percentage of metabarcoding sequences passed quality filtering (79% vs 14%)
- Metabarcoding gave a broader eDNA snapshot (prokaryotes + eukaryotes)

Conclusion

- For methodologies explored:
  - Required minimal equipment
  - Rapid procedure
  - Non-invasive
  - Can be conducted autonomously
- Metabarcoding
  - Proved to be superior to ESS
  - Viably assessed eukaryotic diversity from eDNA seawater samples
- eDNA analyses have great potential for future marine biomonitoring programs