Archaea in Delaware Estuarine Sediments

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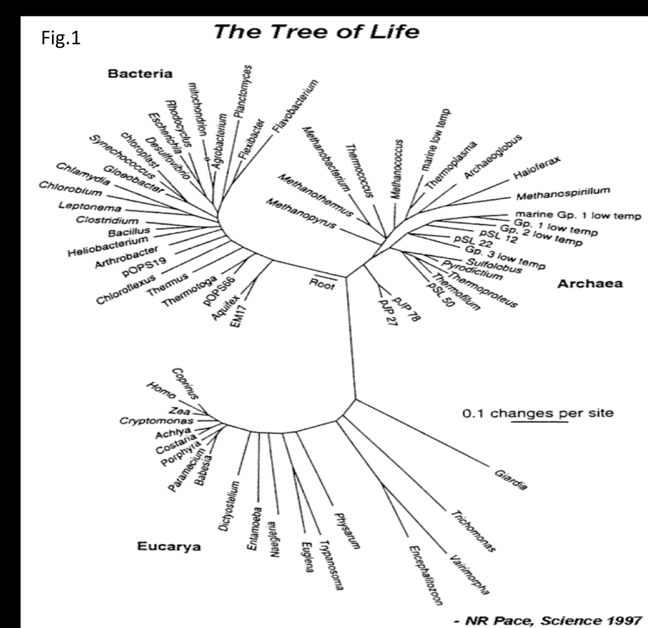
Introduction

Microbes are essential players in global nutrient cycles. In recent years, Archaea, the third domain of life (Fig.1), have received much attention due to their ubiquity, diversity and abundance in terrestrial and marine habitats, both pelagic and benthic. In organic-rich sediments, Archaea have been recognized as active members in the system, however they are mostly uncultivated. There are two main phyla within this domain of Archaea, Euryarchaeota and Crenarchaeota. We expect that Archaea from both phyla will be found in local estuaries, where they will be easy to sample and study. In order to investigate where these organisms live and what they may do, we explored the following questions:

- What species of archaea are present in local Delaware sediments?
- What is their relative abundance in the microbial community?
- Are their distribution patterns associated with salinity, or geochemical indicators?



Methods

Sediment cores were taken from Inland Bays (Indian River Inlet), the Great Marsh, along a salinity gradient in Broadkill River (Fig.2, Table.1) and Rehoboth Bay (Love Creek and Guinea Creek). Each core was sectioned every 2 or 3 cm to create a stratified profile. Each sample went through DNA extraction (MO-BIO PowerSoil DNA Isolation Kit), PCR amplification (Primer sets: A6 and A9, A8F and A915R), cloning (TOPO TA cloning Kit) and Sanger sequencing (GENEWIZ, Inc.) for genomic information. Geochemical analyses include methane, sulfate, porosity sand percentage and other nutrient measurements.

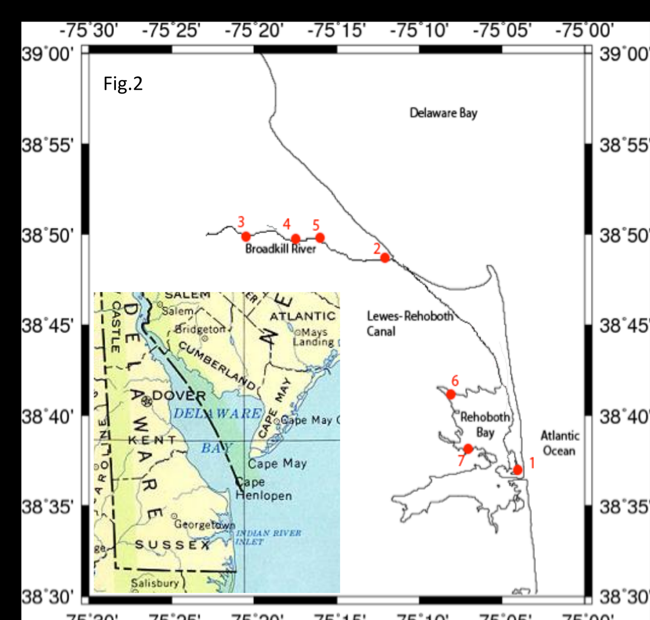


Table.1

No.	1	2	3	4
Sites	Inland Bay	The Great Marsh	Broadkill l River II	Oyster Rock
Salinity	26	35	28	29
No.	5	6	7	
Sites	Broadkill River I	Love Creek	Guinea Creek	
Salinity	14	29	23	

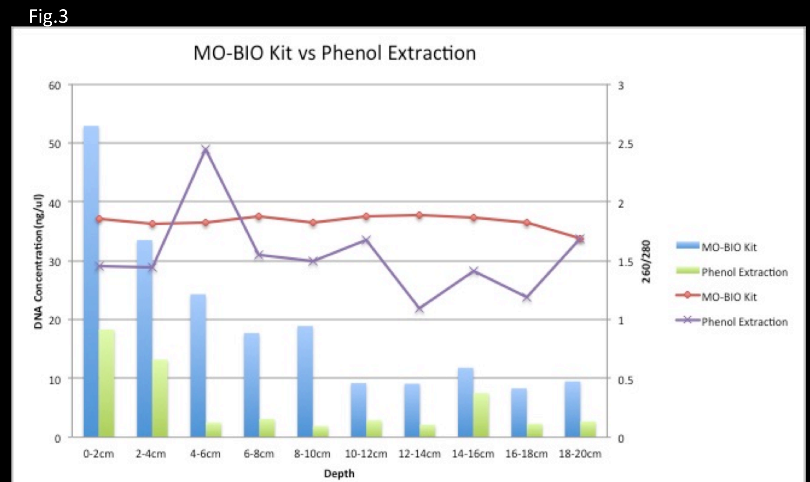


Fig.3: Bars indicate extracted DNA yield, and lines indicate DNA purity. DNA is considered of high purity when 260/280=1.8. These tests showed that DNA quality was best using a MoBio extraction .

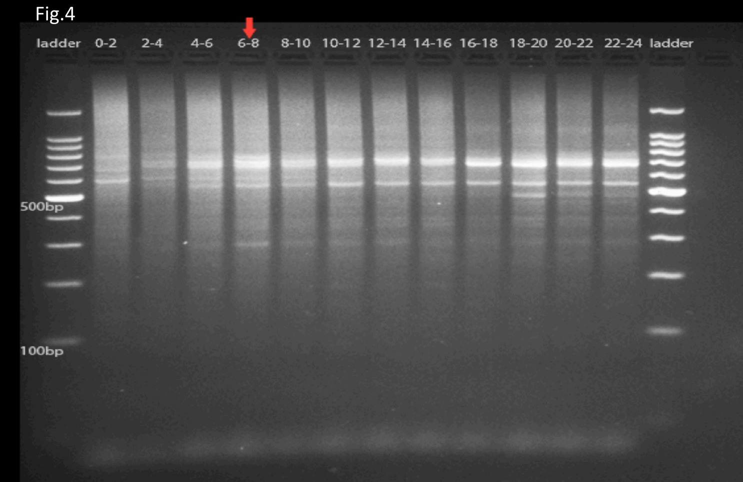
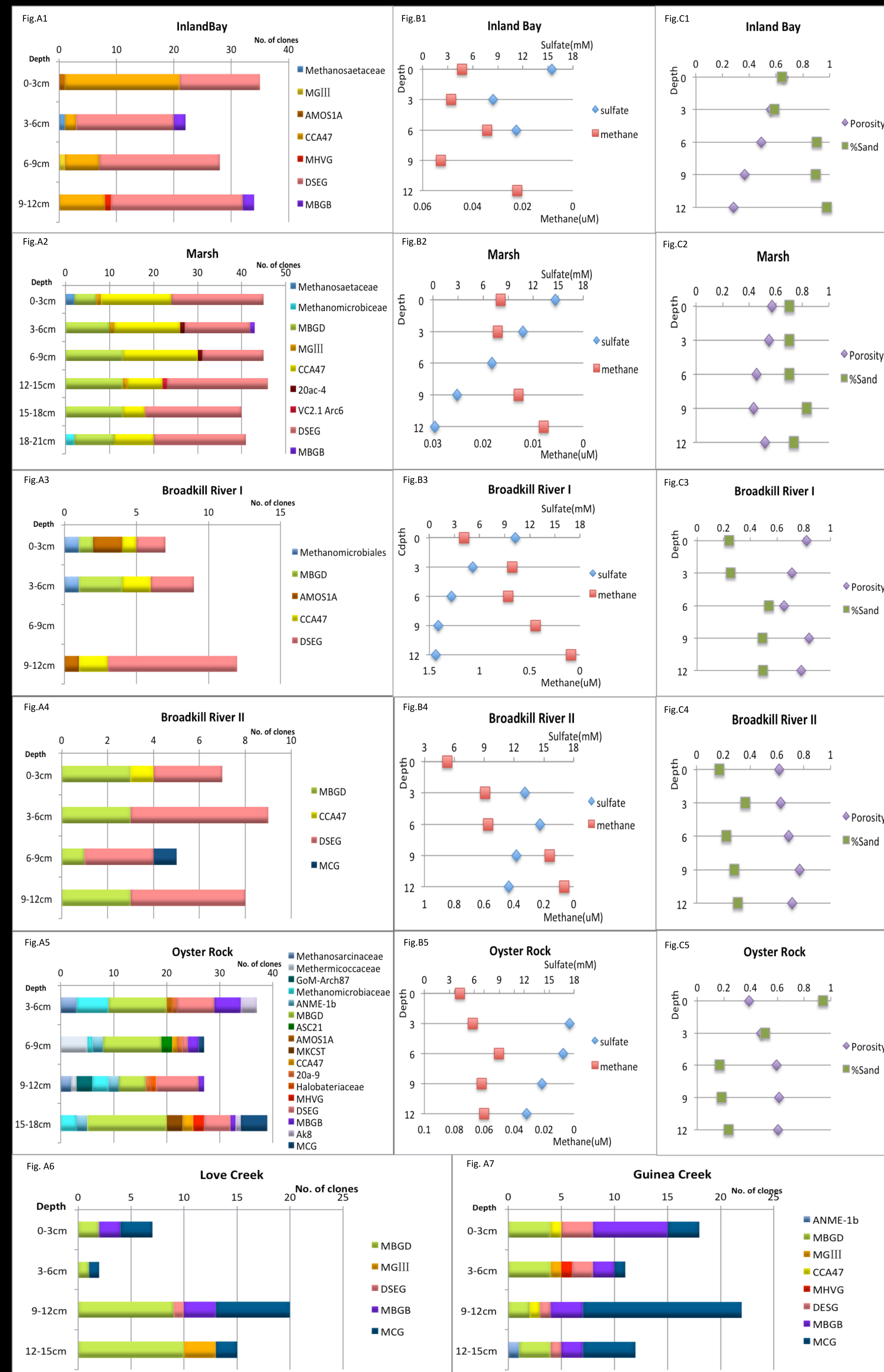


Fig.4: Gel picture showing fingerprint at different depths of one core from Inland Bays. It suggested that populations of Archaea typically stabilize at 6-8 cm below seafloor.

Results

- Initial results of cloning and sequencing showed that the Inland Bay and Great Marsh had a limited diversity of Archaea, but the Broadkill River had a wide diversity of species.
- Euryarchaeota are far more diverse and abundant than Crenarchaeota at these sites. Even though that the MCG archaea are dominating in most estuarine sediments, they are rarely found in Inland Bays or the Great Marsh.
- In the Broadkill River samples, current data suggest that Crenarchaeota, in particular the MCG group, increase in the population as salinity increases.
- Methane and sulfate cycles are present in these samples, there is no clear evidence showing these organisms are associated with sulfate and methane processes at this research level.
- Two additional cores from Rehoboth Bay show a much higher, even dominating percentage of Crenarchaeotal groups, especially the MCG archaea. There are no geochemical data from these two cores at the time being, but will be investigated later to understand the associations.
- Additional nutrient tests, measuring nitrogen species and dissolved inorganic carbon, are currently being run.



Figs A-C: Panel A shows species diversity at different depths of each sample. 47 clones were sequenced to acquire genomic information from samples of Inland Bay, the Great Marsh and Oyster Rock; At two sites from Broadkill River, 10 clones of each were sequenced, and at two sites form Rehoboth Bay(Love Creek, Guinea Creek), 27 clones of each were sequenced. Phylogeny was assigned in ARB. The Panels B and C show methane and sulfate concentrations, porosity and sand percentage at corresponding depth of the same sample. Sulfate samples were obtained from porewater, and some are not valid due to shortage of porewater. No geochemical information of Love Creek and Guinea Creek is available at the time being, but will be investigated in the future. Figures are scaled for best clarity.

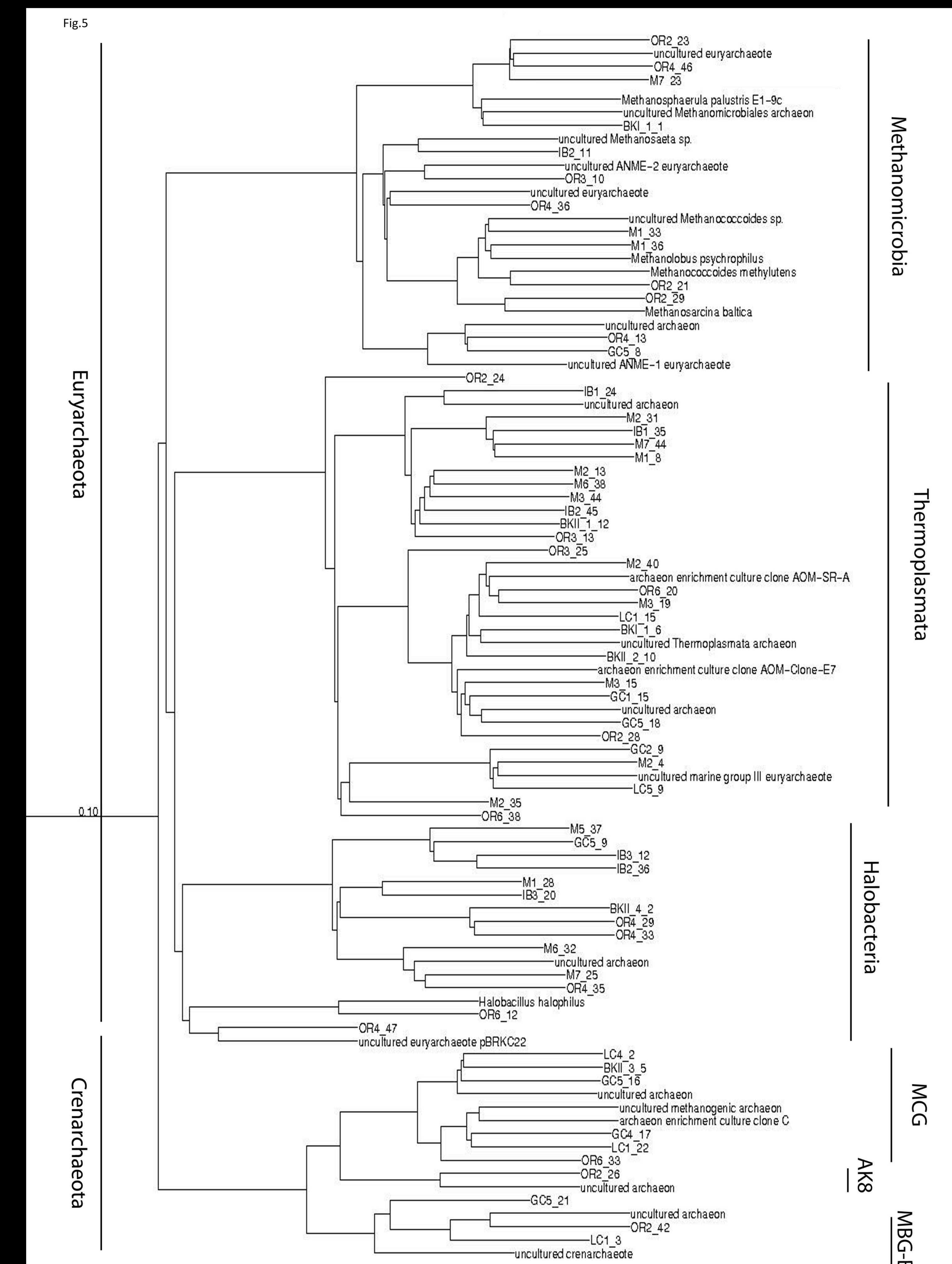


Fig. 5: An ARB tree(Neighbor Joining) showing clones from my samples in relation to representative species from existing database. Name of species indicates locations and depth; for example: OR2_23 means Oyster Rock, depth 2 (3-6cm).

Conclusion and Plans

- Local Delaware sediments house a variety of archaeal groups, many closely related to species from deep, high pressure environments
- Populations differ by site and depth
- Archaeal populations vary with salinity, possibly other nutrients and physical properties
- The local Delaware estuary environment houses readily accessible areas where we can further explore the mysterious lives of sedimentary Archaea
- Future work include examining distribution patterns and chemical associations, and will expand to genomic investigations of uncultured archaeal groups

Acknowledgement

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