**ABSTRACT**

‘Aigarchaeota’ is a candidate phylum of Archaea known only by 16S rDNA gene sequences from cultivation-independent microbial surveys and a single complete genome from Candidatus ‘Caldfarchaeum subterraneum,’ an inhabitant of a subterranean gold mine in Japan. Gene sequences associated with ‘Aigarchaeota’ have been found in a variety of geothermal habitats, however a comprehensive analysis of the phylogeny and distribution of ‘Aigarchaeota’ has not yet been done. Public databases were mined for 16S rDNA gene sequences related to known ‘Aigarchaeota’ and a combination of approaches were used to rigorously define the phylogenetic boundaries of the phylum, investigate its distribution, and design potential ‘Aigarchaeota’ specific primers. Approximately 150 16S rDNA gene sequences affiliated with ‘Aigarchaeota’ were identified, phylogenetic analyses suggested that ‘Aigarchaeota’ belonged to at least three families, to order level groups and at least 13 genus-level groups, and supported the proposed relationship between ‘Aigarchaeota,’ ‘Thaumarchaeota,’ ‘Crenarchaeota,’ and ‘Korarchaeota’ in the so-called ‘TACK’ superphylum. A global distribution was suggested with genus-level group habitat differentiation. Some groups were predominantly terrestrial (1A, 2B, 3, 4 & 5) while other groups were mostly found in marine habitats (6-8). Fifteen strong genus and at least 13 genus-level groups, and supported the proposed relationship between ‘Aigarchaeota,’ ‘Thaumarchaeota,’ ‘Crenarchaeota,’ and ‘Korarchaeota’ in the so-called ‘TACK’ superphylum. A global distribution was suggested with genus-level group habitat differentiation. Some groups were predominantly terrestrial (1A, 2B, 3, 4 & 5) while other groups were mostly found in marine habitats (6-8). Fifteen strong genus and at least 13 genus-level groups, and supported the proposed relationship between ‘Aigarchaeota,’ ‘Thaumarchaeota,’ ‘Crenarchaeota,’ and ‘Korarchaeota’ in the so-called ‘TACK’ superphylum. A global distribution was suggested with genus-level group habitat differentiation. Some groups were predominantly terrestrial (1A, 2B, 3, 4 & 5) while other groups were mostly found in marine habitats (6-8).

**INTRODUCTION**

Despite our knowledge of the diversity of life on earth there are major lineages of microbial life that have never been studied that are referred to as “biological dark matter” or “microbial dark matter” (Marcy et al., 2013). One of these so-called “microbial dark matter” groups was originally described as pSL4 and related gene sequences were later grouped under the name Hot Water Crenarchaeotic Group 1 (HWCG 1) (Barns et al., 1996; Nunoura et al., 2005). A metagenomics study of a microbial mat community from Okinawa Trough revealed a new phylum of archaea referred to as ‘Aigarchaeota’ and further analysis of the genomic data led to the proposition of the phylum ‘Aigarchaeota’ (Nunoura et al., 2005; Nunoura et al., 2011).

**METHODOLOGY**

**PHYLOGENETIC TREE CONSTRUCTION**

- ‘Aigarchaeota’ gene sequences mined from Geobiowork and out-group sequences were aligned in nucleotidise using a 0.5000 character SILVA reference alignment capable of aligning sequences from all three domains of life.
- Alignments were manually curated in BioEdit v7.1.0, trimmed, then saved as Phylogenetic Inference Package (PHYLIP) version 3.69 compatible file.
- A distance matrix was computed using ‘Dnadist’, then used in the ‘Neighbor’ program, which clusters lineages and constructs a tree.
- Sequences were subsequently evaluated at the species (>97%), genus (>95%), family (>90%), order (>85%), and phylum (>80%) level.

**RESULTS**

Figure 1. Neighbor Joining Tree showing 16S rDNA gene phylogenetic relationship between ‘Aigarchaeota’ Groups 1A – 8 and archaeal outgroup, with unsupported nodes identified with boxes. Scale bar represents one substitution per 100 nucleotides.

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**PRIMER EVALUATION**

- ‘Aigarchaeota’ specific qRT-PCR primers were designed by three different programs the SP-Designer, ARB, and Allele ID.
- Primers were classified as forward or reverse and submitted to the Ribosomal Database Project (RDP) Probe Match tool to eliminate primers that were non-‘Aigarchaeota’ specific.
- Primers that did match well with ‘Aigarchaeota’ were reviewed in RDP to determine which genus-level groups were associated with each of the individual primers.
- Primers were analyzed using OligoCalc to ensure appropriate GC content (<50%), melting temperature (60°C), and absence of hairpin formation and potential self-annealing sites.

**REFERENCES**


**CONCLUSION**

This study revealed, for the first time, the phylogenetic and distribution diversity of the novel phylum ‘Aigarchaeota.’ Currently available 16S rDNA gene sequences from global studies suggest that the phylum consists of at least 13 genus-level groups, and that ‘Aigarchaeota’ is situated firmly in the ‘TACK’ superphylum, but exists as a separate phylum that does not possess a phylum-level relationship with the Miscellaneous Crenarchaeotic Group (see Figure 1). Genus-level Group 2A was detected in both terrestrial and marine environments. For contrast, Groups 2B, 3, 4 & 5 and Groups 6-8 are predominant in terrestrial and marine habitats respectively. Groups 1B and 1C were only found in terrestrial habitats, while Group 1D was only found in marine habitats. Potential primers designed in this study range from genus-group specific to a combination of groups allowing for the detection of all potential ‘Aigarchaeota’ defined in this study. It is important to note that these few numbers of marine studies (17) compared to terrestrial studies (34), thus making it difficult to make solid inferences.

**ACKNOWLEDGEMENTS**

This study was supported by the Division of Environmental Microbiology and Microbial Ecosystems of the National Science Foundation (grant number 0820638). We thank the Center for Bioinformatics at the University of Nevada, Las Vegas for access to computing resources.

**THE CENTER**

The Center for Bioinformatics at the University of Nevada, Las Vegas is a multidisciplinary research and education center that provides a unique environment for the development of new ideas and technologies in the field of bioinformatics.

**DISCLAIMER**

This article was written by Gisele Braga Goertz, McNair Scholar, Biological Sciences Major Dr. Brian P. Hedlund, Faculty Mentor, School of Life Sciences

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