



## First insights into enrichment cultures at in situ conditions of hyperthermophilic microorganisms potentially affiliated with the DPANN superphylum

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Archaea DPANN\* superphylum harbors, to date, 12 phyla including Nanoarchaeota and *Candidatus* Aenigmarchaeota (previously described as DHVE-3, DSEG and VAL III). Interestingly, DPANN are detected in a wide range of anoxic or oxygen limited environments such as lakes, marine sediments and hydrothermal systems. Most known core biosynthetic pathways are also absent or incomplete in DPANN Archaea, suggesting symbiotic or parasitic lifestyles. However, much of the knowledge about their ecology, evolution, and putative metabolism mainly relies on metagenomic data. Here we repeatedly enriched two archaea distantly affiliated with *Ca.* Aenigmarchaeota (16S rRNA gene sequence identity <84%) and Nanoarchaeota (16S rRNA gene sequence identity <81%) in continuous cultures. These enrichment cultures were performed from hydrothermal chimney samples and fluids from Snake Pit, TAG and Lucky Strike, hydrothermal sites located on the Mid-Atlantic ridge (BICOSE, HERMINE, MoMARsat 2017, BICOSE 2 oceanographic cruises) using a gas-lift bioreactor under H<sub>2</sub>/CO<sub>2</sub> at 80°C. Cell concentrations increased progressively over several weeks up to ~10<sup>7</sup> cells/mL simultaneously with a relative proportion (≤75%) of these new previously uncultured phylotypes. The enigmatic group of Archaea distantly affiliated with Nanoarchaeota seems to be widespread as it was enriched from all hydrothermal sites, whereas Archaea affiliated with *Ca.* Aenigmarchaeota were only cultured from Snake Pit, suggesting a possible environmental selection. Based on co-occurrence of these phylotypes, several putative archaeal partners were identified. Metagenomic analyses are being performed in order to help to optimize culture conditions for pure (co-)cultures. This ongoing study should help to resolve ecology and life style of these new phylums.

\*an acronym of the names of the first included phyla: Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota and Nanohaloarchaeota.