

Subgroup characteristics of marine methane-oxidizing ANME-2 archaea and their syntrophic partners revealed by integrated multimodal analytical microscopy

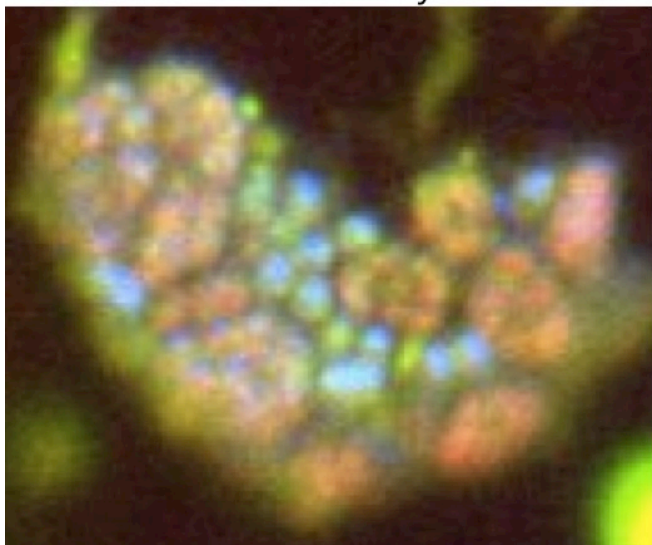
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Phylogenetically diverse environmental ANME archaea and sulfate-reducing bacteria cooperatively catalyze the anaerobic oxidation of methane (AOM) in multi-celled consortia within methane seep environments. To better understand these cells and their symbiotic associations, we applied a suite of electron microscopy approaches including correlative fluorescence *in situ* hybridization –electron microscopy (FISH-EM), transmission electron microscopy (TEM), and serial block face scanning electron microscopy 3D reconstructions (SBEM). FISH-EM of methane seep derived consortia revealed phylogenetic variability in terms of cell morphology, ultrastructure, and storage granules. Representatives of the ANME-2b clade, but not other ANME-2 groups, contained polyphosphate-like granules, while some bacteria associated with ANME-2a/2c contained two distinct phases of iron mineral chains resembling magnetosomes. 3D segmentation of two ANME-2 consortia types revealed cellular volumes of ANME and their symbiotic partners which were larger than previous estimates based on light microscopy. Phosphorous granule containing ANME (tentatively ANME-2b) were larger than both ANME with no granules and partner bacteria. This cell type was observed with up to 4 granules per cell and the volume of the cell was larger in proportion to the number of granules inside it, but the percent of the cell occupied by these granules did not vary with granule number. These results illuminate distinctions between ANME-2 archaeal lineages and partnering bacterial populations that are apparently unified in their capability of performing anaerobic methane oxidation.

Keywords: anaerobic methane oxidation, marine methane seep, microbial ultrastructure, fluorescence *in situ* hybridization, correlative microscopy

Fluorescence *in situ* hybridization



Transmission electron microscopy

