

**Molecular survey of methane-cycling archaea in methane-soaked subsurface sediments
(Guaymas Basin, Gulf of California)**

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The Guaymas Basin is a young marginal rift basin in the Gulf of California characterized by active seafloor spreading, rapid deposition of organic-rich sediments, and steep geothermal gradients. Methane is abundant in the Guaymas Basin hydrothermal sediments and previous studies of surficial Guaymas sediments have yielded a diverse array of methane-cycling archaea. However, their diversity in deep subsurface Guaymas sediments remains unknown. Subsurface sediment samples were selected from eight drilling sites to explore the diversity, depth range and in-situ temperature range of methane-cycling archaea in the Guaymas Basin subsurface. DNA was extracted from near-surface, intermediate, and deeper depths using commercial DNA extraction kits and was PCR amplified using the general and ANME-1 specific primer pairs. *mcrA* was utilized as alternative marker gene to 16S rRNA for the phylogenetic analysis of methane-cycling archaea. A general ANME-1 phylogeny, a methanogen phylogeny, and site-specific phylogenies were constructed. ANME-1 lineages were present in all examined sites, while methanogen lineages were only present in three sites. The methane-sulfate interfaces, which extended into intermediate depths yielded numerous ANME-1 sequences from each examined site. Our work allows for a greater understanding of the diversity of methane-cycling archaea in the Guaymas Basin. Further investigation using matching 16S rRNA datasets and *mcrA* sequences pulled from complete genomes will allow for a comprehensive PCR-based survey of methane-cycling microbial communities in the Guaymas Basin subsurface.