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Palaeo-methanotrophy and carbonate formation: insight from integrated molecular organic, inorganic and fossil 16S rDNA studies

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Anaerobic oxidation of methane (AOM) is the key microbial process, which prevents a significant amount of the greenhouse gas methane to escape to the atmosphere. AOM results in the production of sulfide and the precipitation of carbonate crusts. Although the knowledge about deep-sea cold-venting extreme environments is still insufficient, the immensity of ancient carbonate seep deposits indicates that AOM and related carbonate formation is inherent process in the Earth's history. In this paper we present a study of a fossil methane-related carbonate crust collected from the Kidd mud volcano in the Gulf of Cadiz, NE Atlantic. The idea of the study was to find out the most effective solution for the reconstruction of microbial diversity and biogeochemical conditions shaping ecosystems at the time of the carbonate crust formation. Carbonate chimneys and other carbonate structures occur widespread in the Gulf of Cadiz and probably reflect the presence of cold seeps and associated release of methane in the geological past, possibly in the Early Pleistocene, but it is unclear under what conditions and by which processes these carbonates were formed. Concentrations of microbial lipids, their stable carbon isotope composition, sequences of fossil 16S rRNA genes of anaerobic methanotrophic archaea in combination with mineralogical and carbon and oxygen isotopic composition of carbonate were obtained for seven different horizons of the crust. This combination of organic and inorganic geochemical techniques

with molecular ecological methods gave a consistent view on processes resulting in the formation of the crust and indicated that it took place in two phases and in a downward direction. Archaeal lipid biomarkers and fossil 16S rRNA gene sequence data revealed the dominance of archaeal ANME-2 group and elevated methane partial pressures during the formation of the top part of the crust. The lower part of the carbonate was likely formed in an environment with reduced methane fluxes as revealed by the dominance of fossil remains of ANME-1 archaea. The combination of these methods can be used as an effective tool to reconstruct in unprecedented detail the palaeo-biogeochemical processes resulting in the formation of carbonate fabrics. This interdisciplinary strategy may also be applied for other fossil methane-derived carbonates, generating new concepts and knowledge about past methane-related carbonate systems.