

## Phylogenetic diversity of sediment bacteria from the southern Cretan margin, Eastern Mediterranean Sea

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### Abstract

This study is the first culture-independent report on the regional variability of bacterial diversity in oxic sediments from the unexplored southern Cretan margin (SCM). Three main deep basins (water column depths: 2670–3603 m), located at the mouth of two submarine canyons (Samaria Gorge and Paximades Channel) and an adjacent slope system, as well as two shallow upper-slope stations (water column depths: 215 and 520 m), were sampled. A total of 454 clones were sequenced and the bacterial richness, estimated through five clone libraries using rarefaction analysis, ranged from 71 to 296 unique phylotypes. The average sequence identity of the retrieved Cretan margin sequences compared to the > 1,000,000 known rRNA sequences was only 93.5%. A diverse range of prokaryotes was found in the sediments, which were represented by 15 different taxonomic groups at the phylum level. The phylogenetic analysis revealed that these new sequences grouped with the phyla Acidobacteria, Planctomycetes, Actinobacteria, Gamma-, Alpha- and Delta-proteobacteria. Only a few bacterial clones were affiliated with Chloroflexi, Bacteroidetes, Firmicutes, Gemmatimonadetes, Verrucomicrobia, Nitrospirae, Beta-proteobacteria, Lentisphaerae and Dictyoglomi. A large fraction of the retrieved sequences (12%) did not fall into any taxonomic division previously characterized by molecular criteria, whereas four novel division-level lineages, termed candidate division SCMs, were identified. Bacterial community composition demonstrated significant differences in comparison to previous phylogenetic studies. This divergence was mainly triggered by the dominance of Acidobacteria and Actinobacteria and reflected a bacterial community different from that currently known for oxic and pristine marine sediments.

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## Introduction

Bacteria are accountable for most of the benthic biomass and it is well known that they play a significant ecological and biogeochemical role in marine ecosystems by regulating the transformation of major bioactive elements (i.e. carbon, nitrogen, phosphorus, oxygen and sulfur) [17,20,45], and by affecting the degradability of organic matter [1]. Although the fundamentals of physical and chemical oceanography have long been established, comprehensive studies describing microbial diversity and processes are only now emerging [18]. With the rise of molecular genetic tools, it has become apparent that we only know about a very small fraction of the diversity of the microbial world. Most of this unexplored microbial diversity seems to be hidden in the large number of yet uncultured bacteria. The most common of these techniques uses ribosomal gene sequences as indicators of bacterial diversity [4,47].

The characterization of natural microbial communities in deep-sea sediments has been one of the major challenges in the field of microbial ecology. Considering that 71% of the earth's surface has an average depth of 3800 m, deep-sea environments have attracted much interest as niches of microbial life with considerable exploitation potential. The deep eastern basin of the Mediterranean Sea is considered to be one of the world's

most oligotrophic areas and is characterized by an overall nutrient deficit [23,26]. As a result, only small amounts of organic matter reach the sea floor through the water column, resulting in low bacterial community growth and abundance [16,17]. In the open ocean, the organic matter is predominantly provided by primary production in the photic zone. In contrast, continental margins represent one of the highest production zones of the ocean and they receive organic inputs mainly from the continents, thus reshaping benthic communities [33]. Furthermore, organic matter can be transported from the continental shelf to the adjacent deep environments through specific pathways, such as submarine canyons (e.g. [12,35,39]). The southern Cretan margin (SCM) is ideal for studying such processes since two major submarine canyons are found in this area, namely the Samaria Gorge and the Paximades Channel that debouch into a number of deep basins [2]. However, despite its importance regarding organic matter transportation and recycling, little is known about the microbial diversity of this type of ecosystem.

The present study aimed at investigating the bacterial diversity and composition of the unexplored SCM in the Eastern Mediterranean Sea by the construction of five large 16S rDNA clone libraries. Three main deep-sea basins located at the mouth of the two submarine canyons (Samaria Gorge and Paximades Channel [2]) and an adjacent slope system, as well as two additional

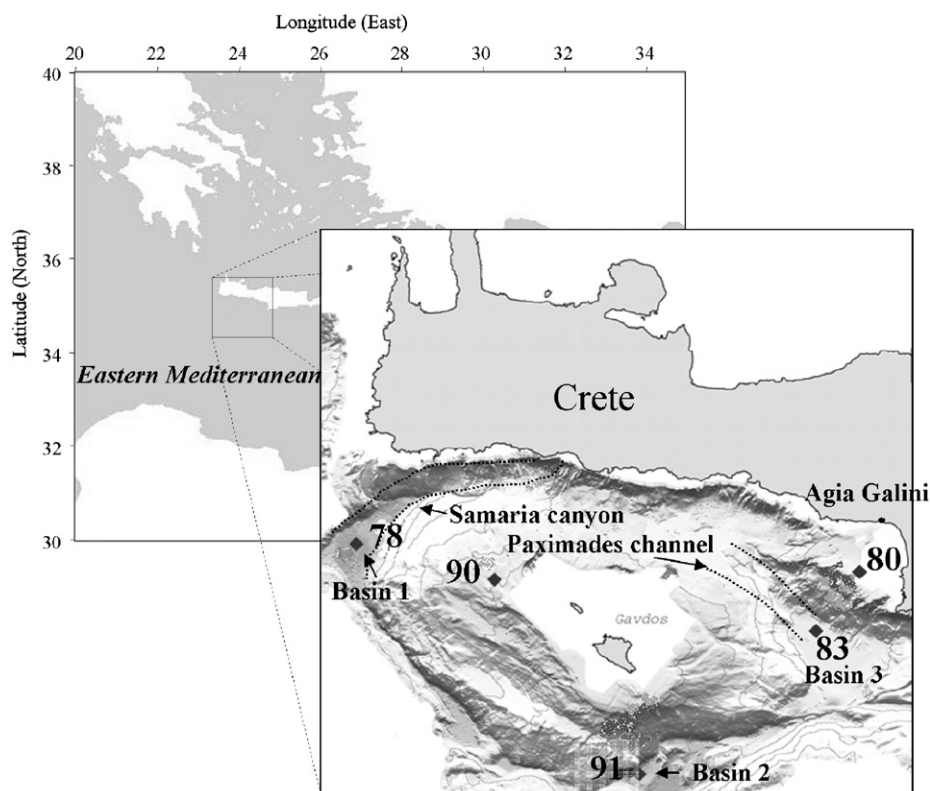


Fig. 1. Map showing the positions of the stations sampled.

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