SYMBIOTIC CALCIFYING BACTERIA ACROSS SPONGE SPECIES AND OCEANS

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Abstract
In this study, we pursued to analyze the microbiome of phylogenetically and geographically distant sponge species, which harbor calcareous spherules of a purported bacterial origin. The analyzed sponges were spread along the circumtropical belt. The results showed the dominance of proteobacteria in all the species examined but two. The targeted calcibacterium was retrieved in *H. columella, H. arabica, C. viridis* and *C.alloclada*. However, other bacteria also produced calcareous spherules in *Hemimycale sp*. (Mediterranean) *Crella cyathophora* (Red Sea), Cinachyrella sp. (Caribbean) what indicates that calcifying symbioses between sponges bacteria are widespread. The results reinforce the hypothesis on bacteria involvement, through symbiotic associations, in skeletonization processes of early Metazoans.

Keywords: Mediterranean Sea, Red Sea, Symbiosis, Bacteria, Porifera

Introduction
Sponges are important members of benthic ecosystems in terms of both abundance and diversity [1]. They harbor huge amounts of symbiotic bacteria within their tissues, which have been reported to play several roles in nutrient cycles, production of secondary metabolites or sponge protection from predators [2]. The latter function has been recently documented for an abundant endosymbiotic bacterium found in the mesohyle of the Atlanto-Mediterranean sponge *Hemimycale columella*. The bacterium calcifies within vacuoles of a particular sponge cell (calcibacteriocyte) forming a calcareous envelope, which is easily visible through light microscope [3]. Hundreds of calcibacteria envelopes accumulate at the sponge periphery forming a kind of rudimentary exoskeleton. Recently this bacterium has been identified as an alphaproteobacterium, representing up to 67% of the sponge microbiome [4]. The aim of this study was to seek out the presence of calcibacteria in sponges other than *H. columella*, which have been observed through light microscope to contain calcareous spherules in high amounts and to compare the microbial communities of these sponge species across Seas.

Methods
The spherule bearing sponges analyzed were: *Hemimycale columella*, *Hemimycale sp.*, *Cliona viridis* (Mediterranean Sea), *Hemimycale arabica*, *Crella cyathophora* (Red Sea), *Crella cyathophora* (Indian Ocean), and *Cinachyrella sp.* and *Cinachyrella alloclada* (Caribbean Sea). Three individuals per species were sampled by SCUBA diving and tag-pyrosequenced using a 454 Roche platform. Data obtained from pyrosequencing were analyzed using QIIME 1.4.0 pipeline [5].

Results and Discussion
The microbiome of the sponges harboring calcareous spherules was species-specific. Proteobacteria was the dominant (relative abundances ≥70%) phylum in all the species analyzed but *Cinachyrella sp.* and *C. alloclada*.

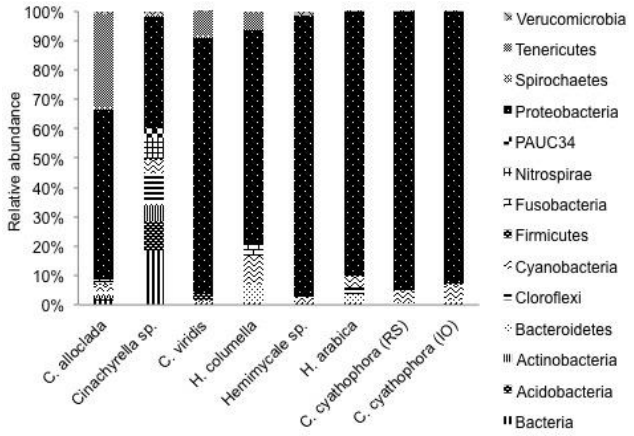

Fig. 1. Relative abundance of bacteria Phyla in the sponge species studied (OTUs with relative abundances ≤1% in a given sponge species were removed).

Furthermore, the calcibacterium of *H. columella* proved not to be species-specific but has also been recorded in *C. alloclada, H. arabica* and *C. viridis*. However, intracellular calcification is also produced by different bacteria in *Hemimycale sp.* (Mediterranean) *Crella cyathophora* (Red Sea) and *Cinachyrella sp.* (Caribbean) what indicate that the symbioses between sponges and calcifying bacteria are more widespread than previously believed. This particular type of symbiosis, which was totally unknown until 2012 [3], appears to be widespread among warm water sponges. These results support the hypothesis on the involvement of intracellular bacteria in the formation of calcareous skeletons in Early Metazoans.

References
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