FIRST REPORT OF *LEPTOLYNGBYA* (CYANOBACTERIA) SPECIES ASSOCIATED WITH MARINE SPONGES IN THE AEGEAN SEA

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Abstract
Sponge associations with cyanobacteria have been poorly investigated in the eastern Mediterranean. Herein, the marine sponges *Acanthella acuta, Chondrilla nucula, Dysidea avara,* and *Petrosia ficiformis* from the Aegean Sea were found associated with cyanobacteria of the genus *Leptolyngbya,* using culture-dependent methods. Four *Leptolyngbya* strains with distinct morphology and phylogeny, were isolated.

Keywords: Porifera, Symbiosis, Systematics, Aegean Sea, Algae

Introduction The association between cyanobacteria and sponges is thought to be one of the oldest microbe-metazoan interactions [1]. To date, cyanobacteria symbionts have been recorded in at least 100 sponge species [2]. Cyanobacteria species involved in such symbioses belong to the genera *Synechococcus, Synechocystis, Aphanocapsa, Oscillatoria, Cyanobacterium,* and *Prochlorococcus* [3]. Moreover, *Halomicronema* and *Leptolyngbya* species have been recently found in association with the sponge *Petrosia ficiformis* [4,5]. Although cyanobacteria may comprise 25-50% of sponge volume, we still lack a clear picture of their diversity and ecological role as sponge symbionts [2] especially in the eastern Mediterranean Sea. The present study is part of a broader research aiming to investigate the diversity of cyanobacteria associated with sponges in the Aegean Sea, on which no information exists.

Material and Methods Sponge samples were collected by Scuba diving at depths between 5-20 m, in October 2014. An 1 cm^3 portion of each sponge sample was briefly rinsed in 70% ethanol and rapidly transferred to sterile sea water. Each tissue was cut into thin sections and homogenised. Serial dilutions of the suspension were prepared in liquid MN medium. The cultures were incubated at 22 ± 1.0 ºC under white fluorescent light and a light cycle of 12:12 hours. Morphological examination of cyanobacteria isolates were performed using a Zeiss Axio imager z2 microscope. The 16S rRNA gene was amplified from genomic DNA using cyanobacteria specific-primers 106F (5?-CGG ACG GGT GAG TAA CGC GTG-3?) and 23S30R (5?-CTT CGC CTC TGT GTG CCT AGG-3?). Partial 16S rRNA sequence data were obtained from cyanobacteria strains and compared with other sequences available in GenBank using Blastn. The phylogenetic tree was constructed by the maximum likelihood method using Mega 6.06 [6], applying a GTR + G + I model of nucleotide substitution.

Results and Discussion Four cyanobacteria strains (denoted AUTH 0915, 1215, 1015, and 1115) were isolated from the sponges *P. ficiformis, C. nucula, D. avara,* and *A. acuta*. *Morphology*. Filaments were densely and irregularly entangled, joined in clusters. Sheaths were mostly diffluent, rarely distinct and colourless. Cells were longer than wide in three stains (AUTH 0915, 1015, 1215), whereas in AUTH 1011 strain they were shorter than wide. Trichomes had pink or pale purple colour. The four isolates exhibited all the typical features of *Leptolyngbya* [7] but none of them had all the morphological characters of any species of the genus. *Phylogenetic analysis*. Strains AUTH 0915, 1015, and 1115 formed a separate subcluster close to the marine *L. ectocarpi* cluster, whereas strain AUTH 1215 was placed outside this clade (Fig. 1). Strains AUTH 0915 and 1015 showed 98% pairwise sequence similarity with *L. ectocarpi* strains, whereas strains AUTH 1115 and 1215 showed 97% similarity. This is the first record of filamentous cyanobacteria (*Leptolyngbya*) living in association with the sponges *C. nucula, D. avara,* and *A. acuta*. Previously, *Leptolyngbya*-likestrains have been isolated from the sponge *P. ficiformis* [4], one of them being the novel species *Halomicronema metazoicum* [5]. The observed differences in morphology, phylogeny, and ecology suggest that the *Leptolyngbya* strains isolated in this study could be new species, but further investigation is required. For the time being, the strains were assigned to the taxon *Leptolyngbya* sp.

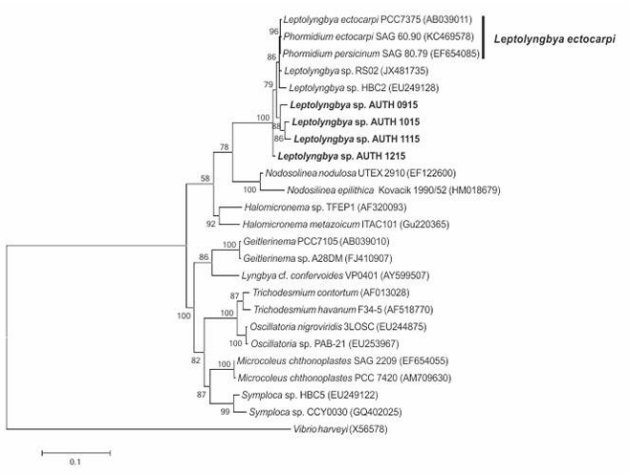

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences and reconstructed using the maximum–likelihood (ML) analysis. Numbers above branches indicate the bootstrap value (as percentages of 1,000 replications). Strains of the present study are indicated in bold, GenBank accession numbers are indicated in brackets. Bar represents 0.1 nucleotide substitutions per site.

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Marine sponges are sessile, benthic dwellers in the reef environment and harbor a high abundance of symbiotic microorganisms that can account for up to 40% of their body volume (1). High-throughput sequencing of bacterial 16S ribosomal RNA genes has revealed the enormous diversity and stability of the sponge bacterial community, which is distinct from that of the surrounding environment (1.Â Symbiotic cyanobacteria can transfer photosynthetic carbohydrates to spongesÂ (C) Enrichment culture of the filamentous cyanobacterial Leptolyngbya isolated from sponge I. stroblina tissue. (Scale bar: 1 Î¼m.) Download figure. Candidatus Poribacteria were first identified in the marine sponge Aplysina aerophoba more than 14 years ago [1], but have never been successfully isolated in laboratory culture.Â Average sizes for genome bins of sponge-associated Poribacteria reported as more than 90% complete by CheckM were actually slightly larger (5.4 Â± 0.69 MB, n = 10) than those from the Tara Oceans dataset (5 Â± 0.28 MB, n = 9), although this difference was not statistically significant (p-value = 0.18, two-tailed t-test).Â Tara Oceans genomes are italicized, with geographic abbreviations ARS, Arabian Sea; RS, Red Sea; MED Mediterranean Sea; NAT, North Atlantic; SAT, South Atlantic; NP, North Pacific; SP, South Pacific. Outgroup abbreviation RB_SH1 indicates Rhodopirellula baltica strain SH1. Cyanobacterial diversity associated with sponges remains underestimated, though it is of great scientific interest in order to understand the ecology and evolutionary history of the symbiotic relationships between the two groups. Of the filamentous cyanobacteria, the genus Leptolyngbya is the most f â€¦Â Of the filamentous cyanobacteria, the genus Leptolyngbya is the most frequently found in association with sponges as well as the largest and obviously polyphyletic group. In this study, five Leptolyngbya-like sponge-associated isolates were investigated using a combination of molecular, chemical, and morphological approach and revealed a novel marine genus herein designated Leptothoe gen. nov. Cyanobacteria, in the marine environment, have been reported to live in association with a remarkable variety of hosts (e.g., fungi, ascidians, corals, and protists) [10], while the association formed with sponges has significantly attracted research interest from a biotechnological perspective (among others) in the last decades.Â Our recent research on sponge-associated cyanobacteria in the North Aegean Sea [15] resulted in the isolation of 15 cyanobacteria strains [15 , 16] and showed novel cyanobacteria diversity. Indeed, the investigation of five Leptolyngbya -like sponge-associated isolates... Although a few species each of carnivorous and solitary sponges exist, most sponges symbiotically inhabit coral reefs, where they participate in nutrient cycles with organisms in the coral reef. Endosymbiotic fungi, bacteria and viruses extensively and obligately co-habitate with marine sponges. These organisms participate in the nutrient cycle of the sponge as well as in the production of the protective, chemically unusual secondary metabolites. It is these bioactive molecules that are the primary subject of natural product discovery in sponges.