

## Further findings from Daveli Cave (Attica, Greece) enhancing the establishment of the genus *Oculatella* (Pseudanabaenaceae, Cyanobacteria)

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### Abstract

In this study, cyanobacterial assemblages were collected from the limestone cave Daveli (Attica, Greece) during an annual survey (2010–2011). Four sampling areas were selected from the cave entrance inwards, and the main abiotic parameters (PAR, T, RH) were measured at each site. A portion of the collected material was fixed with formaldehyde solution and another part was maintained alive for culturing and microscopic observations. XRD analysis was applied for analyzing the chemical composition of the limestone substrate. Microscopic observation (LM) revealed the presence of a wealth of Cyanobacteria including a brownish-violet colored filamentous morphotype with a large granulated ‘ocellus’ in the apical cell, features resembling the newly (2012) established genus *Oculatella* of the family Pseudanabaenaceae. This morphotype was isolated in unialgal cultures for a further morphological (SEM and TEM), molecular (16S rRNA) and biochemical analysis (FAME). The 16S rRNA sequence generated from the specimen revealed a 99% similarity with *Leptolyngbya* sp. VRUC135 (GenBank–X84809), a strain now recognized as the type species (*Oculatella subterranea*) of *Oculatella*. Taking a step forward, we proceeded to the determination of fatty acid composition by Gas Chromatography which revealed the dominance of palmitic acid (16:0) in all isolates followed by palmitoleic acid (16:1), linoleic acid (18:2), and oleic acid (18:1), while tridecylic acid (13:0), myristic acid (14:0), isomers of C<sub>15</sub>, C<sub>17</sub>, C<sub>20</sub> and C<sub>22</sub>, stearic acid (18:0), linolenic acid (18:3), as well as an unknown fatty acid were found in traces. These new findings corroborate the establishment of the new genus *Oculatella* and provide information on the fatty acid composition of the species *Oculatella subterranea*.

### Introduction

Cyanobacteria represent an ancient group of oxygenic, photosynthetic prokaryotes with considerable morphological diversity, widespread all over the world (Garcia-Pichel 2009). The long evolutionary history of Cyanobacteria is considered to have contributed to their successful colonization in a variety of habitats (i.e. cold deserts, hot deserts, hot springs, subterranean ecosystems including caves, etc) implying a wide ecological tolerance (Whitton & Potts 2012).

The immense diversity of Cyanobacteria refers not only to morphological variability and habitat preferences but also to a variety of produced bioactive metabolites (including compounds with antibacterial, anticancer, antiviral, cytotoxic, antifungal, etc. activities) as well as lipids and fatty acids (Burja *et al.* 2001, Singh *et al.* 2005, Ramadan *et al.* 2008, Abed *et al.* 2009, Rastogi & Sinha 2009, Tan & Goh 2009, Jones *et al.* 2010, Nunnery *et al.* 2010, Thi Anh Tuyet 2010). Furthermore, Cyanobacteria represent a rich source of the nutritionally important long chain polyunsaturated fatty acids (Singh *et al.* 2002).

Within the phylum of Cyanobacteria the traditional classification system is based on morphological characters as observed in field samples as well as in cultured material (Komárek & Anagnostidis 2005). However, many new data based on ultrastructural characteristics and on genetic information imply the need of revision of the existing classification system (Hoffmann *et al.* 2005, Johansen & Casamatta 2005, Komárek 2006, 2010). The combination of both morphological and molecular data is being used today for determining the taxonomic position of Cyanobacteria applying what is mentioned as ‘polyphasic approach’ in the cyanobacterial systematics (e.g. Lamprinou *et al.* 2011, 2012, 2013, Komárek *et al.* 2013, Roldán *et al.* 2013, Mühlsteinová *et al.* 2014a, b). Furthermore, the differences observed in the fatty acid composition of Cyanobacteria can be used as a taxonomic tool (Kenyon 1972, Kenyon *et al.* 1972,

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