Identification of sulfur-oxidizing *Thiothrix* bacteria on microcrustaceans from the sulfidic groundwaters of Mangalia (southeastern Romania)

Filip Paul Boancă[‡], Claire Audrey Chauveau[§], Jean-François Flot[§,|], Maria Mirabela Pop[‡], Sanda Iepure[‡,¶]

‡ "Emil Racovita" Institute of Speleology, Cluj-Napoca, Romania
§ Université libre de Bruxelles, Brussels, Belgium
| Interuniversity Institute of Bioinformatics in Brussels – (IB)², Brussels, Belgium
¶ Romanian Institute of Science and Technology, Cluj-Napoca, Romania

Corresponding author: Filip Paul Boancă (filip.boanca@yahoo.com)

Abstract

Movile Cave, located in southeastern Romania close to the Black Sea, is one of the most remarkable diversity hotspots worldwid with 52 species of invertebrates of which 37 are endemic (Brad et al. 2021). Due to the high concentration of hydrogen sulfide in its water, the primary production of organic matter in Movile Cave relies almost entirely on the chemoautotrophic activity of microorganisms, notably sulfur-oxidizing gammaproteobacteria belonging to the genus *Thiothrix*. In the presence of oxygen, these filamentous bacteria can oxidize hydrogen sulfide and reduce it into various sulfidic compounds, generating energy in the process. In sulfidic ecosystems, *Thiothrix* bacteria are frequently found free-living but also as epibionts or ectosymbionts growing on other organisms, such as amphipods (Flot et al. 2014). However, it is unclear whether *Thiothrix* bacteria also grow on microcrustaceans such as copepods or ostracods, of which several species are known from the sulfidic mesothermal aquifer of Mangalia, where Movile Cave is located. To find it out, we combined DNA sequencing using the reference bacterial 16S ribosomal RNA gene with morphological observations (including fluorescence microscopy). Our results reveal that *Thiothrix* bacteria are indeed present on microcrustaceans from Movile Cave and surrounding wells in the Mangalia region, highlighting the versatility of *Thiothrix*-crustacean associations in sulfidic ecosystems. This is the first report of an association between *Thiothrix* and groundwater microcrustaceans, and the second report of an association between *Thiothrix* and a nonmarine ostracod (Khalzov et al. 2021).

Keywords

Movile Cave; Mangalia groundwater; sulfur-oxidizing bacteria; subterranean biodiversity; groundwater ecosystems; crustacean ectosymbionts

Presenting author

Filip Paul Boancă

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Conflicts of interest

The authors declare no conflict of interest.

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