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Exploring the crustaceans world in the sulfidic mesothermal aquifer from Mangalia, Romania (Southern Dobrogea, Romania)

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The Mangalia town region in Southern Dobrogea, Romania, conceals dark, isolated, and sulfide-rich overlapping aquifers hiding intriguing and complex hydrogeological characteristics (Marin & Nicolescu, 1993; Sarbu, 2000). This unique ecosystem is sustained by a high level of primary production through chemosynthesis, mainly supported by chemoautotrophic bacteria and protozoa that derive their energy from chemical reactions with inorganic molecules such as hydrogen sulfide, elemental sulfur, and ammonia under low oxygen or anaerobic conditions (Sarbu *et al.*, 1994, 1996; Engel, 2007; Kumaresan *et al.*, 2014; Sarbu *et al.*, 2019). The Upper Sarmatian sulfidic mesothermal aquifer, the focus of the current study, which is accessible through the old hand-dug wells (sulfidic and non-sulfidic water), and the Movile Cave provide a suitable habitat for a diverse community of cyclopoid crustaceans (eight species) with a preponderance of stygobites (75%), from the following genera: *Acanthocyclops* Kiefer, 1927, *Diacyclops* Kiefer, 1927, *Tropocyclops* Kiefer, 1927, and *Eucyclops* Claus, 1893. The spatial distribution of the identified species does not indicate a distinct affinity for a specific type of water, sulfidic or non-sulfidic.

The primary production in Movile Cave is mainly dependent on the activity of sulfur-oxidizing bacteria, particularly sulfur-oxidizing filamentous gammaproteobacterial species of the genus *Thiothrix* (Sarbu *et al.*, 1994; Chen *et al.*, 2009).

Thiothrix bacteria can be found either as free-living bacteria or as epibionts or ectosymbionts attached to other animals, such as amphipods, in marine or continental sulfidic habitats (Gillan & Dubilier, 2004; Dattagupta *et al.*, 2009; Bauermeister *et al.*, 2012; Flot *et al.*, 2014; Borko *et al.*, 2019). The first *Thiothrix*-amphipod symbiosis in a sulfidic groundwater habitat was reported in the Frassasi Caves system, Italy, involving three *Niphargus* amphipod species (Bauermeister *et al.*, 2012; Dattagupta *et al.*, 2009). Later, the same *Niphargus*–*Thiothrix* association was also discovered in aquifers located 1200 kilometers apart, in Movile Cave and the surrounding aquifer in Mangalia, as well as in other groundwaters in the Dobrogea region (Limanu, Dulceşti, Doi Mai, Vama Veche) (Flot *et al.*, 2014). Thus, this suggests that they could be widespread in continental sulfidic and non-sulfidic aquifers in Europe, especially since a similar association was also observed in Melissotrypa Cave from Greece, where *Niphargus gammariformis* seems to have established an interaction with *Thiotrix* filamentous bacteria (Flot *et al.*, 2014; Borko *et al.*, 2019). Furthermore, *Thiothrix* bacteria has been found to be also present on cyclopoid or ostracod microcrustaceans from both the Movile Cave and the top layer mezotermal aquifer in Mangalia, especially since this association could potentially provide advantages for the species involved (Chauveau *et al.*, in prep).

Applying the sequencing of 16S ribosomal

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DNA *Thiothrix* genes, we show that bacteria of the genus *Thiothrix* are present on *Eucyclops graeteri scythicus*, *Tropocyclops prasinus* (Cyclopoida, Copepoda) and *Pseudocandona movilaensis* (Ostracoda) thriving in the aquifer of the Mangalia town and Movile Cave, highlighting the versatility of such *Thiothrix*-crustacean associations in sulfidic groundwaters. This study signals the first discovery of such associations between ostracod and cyclopoid crustaceans and *Thiothrix* in sulfidic groundwater and a cave ecosystem worldwide, expanding our understanding of symbiotic relationships between crustaceans and *Thiothrix* (Chauveau *et al*., in prep).

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