

## 4.6 Bio- and geodiversity of vermiculations from

### Pertosa-Auletta Cave (southern Italy)



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FONDAZIONE



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#### Introduction

Caves are largely unknown environments, hosting a wide diversity of extremophile microorganisms, highly specialized and adapted to their prohibitive conditions (1). Vermiculations, that are thin, irregular and discontinuous deposits of incoherent particles with several morphologies, colors and size, commonly found on the walls and ceilings of natural or artificial caves worldwide (2, 3), are considered “life hotspots”. The role of cave biota in the formation of these enigmatic structures is still largely unexplored (4).

The aim of this study, funded by MIdA Foundation (Italy), was to investigate the microbiological and physico-chemical properties of vermiculations from Pertosa-Auletta Cave (Campania, southern Italy), in order to clarify the mechanisms involved in their formation and to define the factors affecting their diversification.

#### Study area

Pertosa-Auletta Cave is located in the northeastern zone of the Alburnian Massif (Fig. 1), one of the most important karst area in Campania region, evolving in a 1500 m thick Mesozoic to Cenozoic carbonate sequence (5). It is ~ 3 km long and consists of 3 principal parallel branches, plus a perpendicular one that links the two southernmost trails (Fig. 2). The eleven samples analyzed (Fig. 2) were collected paying great attention to avoid impacts on the wall. Sterile and plastic materials were used, respectively, for microbiological and geochemical analyses (Fig. 3).

#### Results and discussion

The vermiculations of Pertosa-Auletta Cave are characterized by different morphologies and colors (Fig. 4). X-ray diffraction and energy dispersive X-ray analysis indicated calcite as the dominant mineral of the vermiculations studied, whereas clay minerals were almost absent, putting in doubt one of the most common definition regard vermiculations as “clayey-silty” deposits. Elemental analysis showed wide variations in C, organic C, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, N, Na, Ni, P, S, Ti, V and Zn concentrations among vermiculations. Applying the most innovative techniques in molecular biology, only Prokaryotes were observed. In particular, 16S rRNA gene-specific next generation sequencing analysis showed that Proteobacteria (48.0%) was the most abundant phylum in the Bacteria domain, followed by Acidobacteria (11.6%), Actinobacteria (7.1%), Nitrospirae (5.8%), Firmicutes (4.3%), Planctomycetes (3.2%), Chloroflexi (1.9%) and Gemmatimonadetes (1.1%). Archaea (0.1%) and a significant percentage of unclassified microorganisms (13.1%) were also observed (Fig. 5). Confocal Laser Scanning Microscopy and Field Emission Scanning Electron Microscopy images confirmed the NGS results, showing the presence of biological evidences (Fig. 6 and 7A, B, D) and biogenic calcite (Fig. 7C), clearly indicating microbial activities.

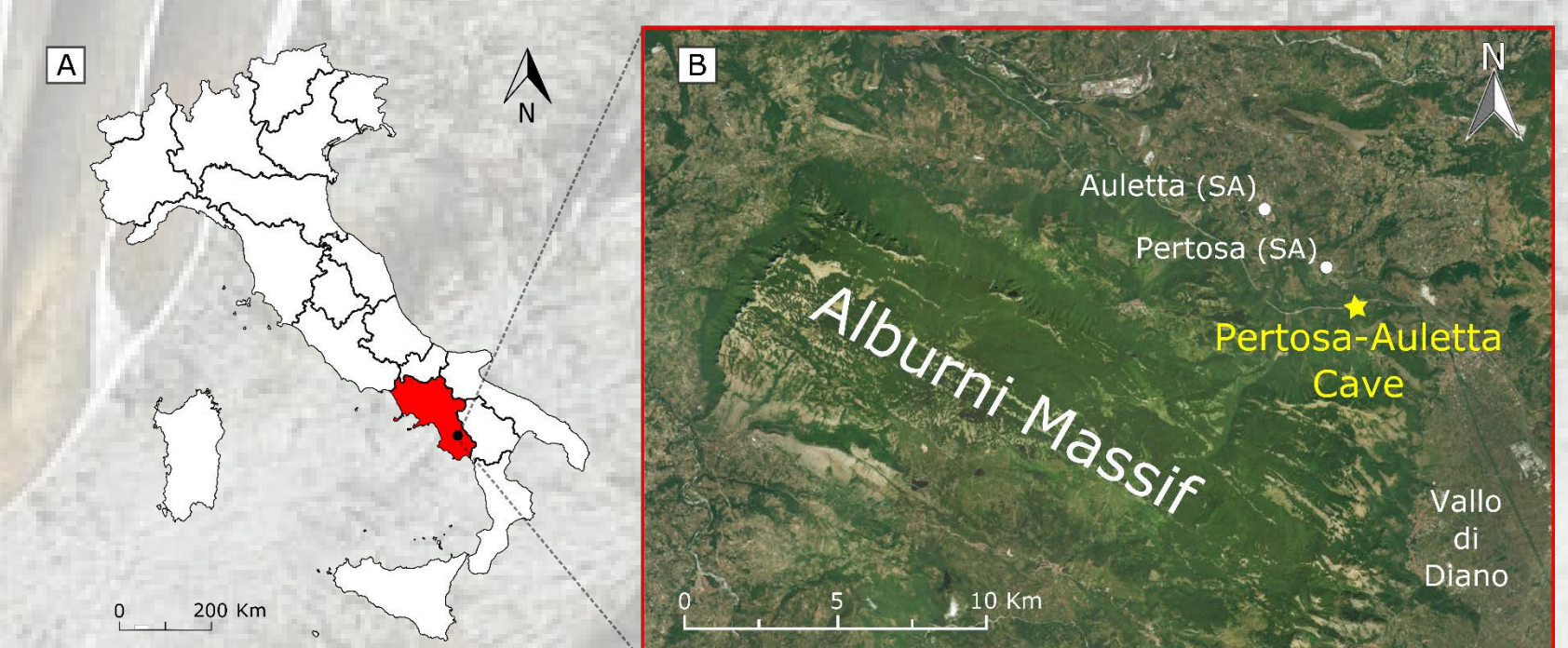


**Figure 1** - Geographical setting of Pertosa-Auletta Cave. A. National overview with Campania region in evidence; B. Local overview.

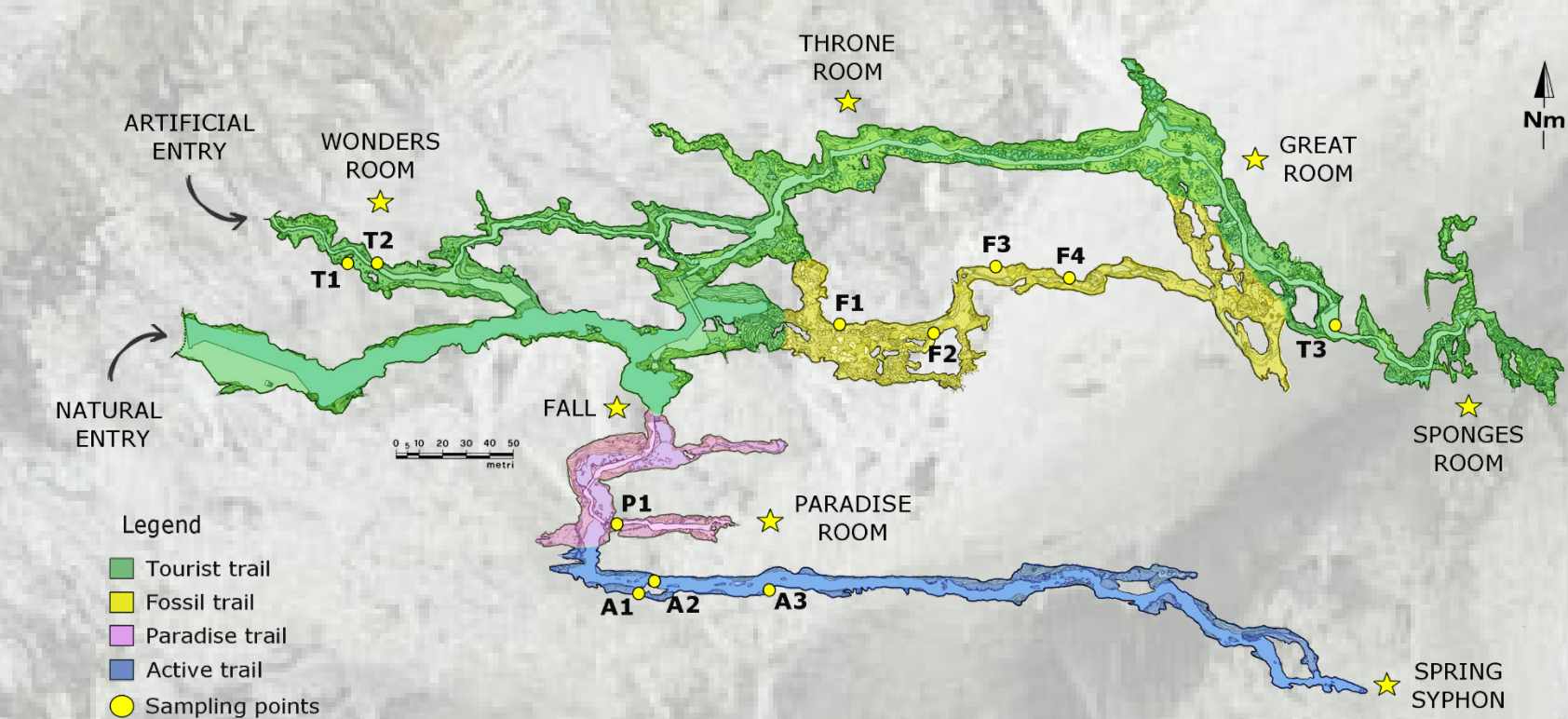


**Figure 2** - Map of Pertosa-Auletta Cave with the sampling points in the 4 different trails.

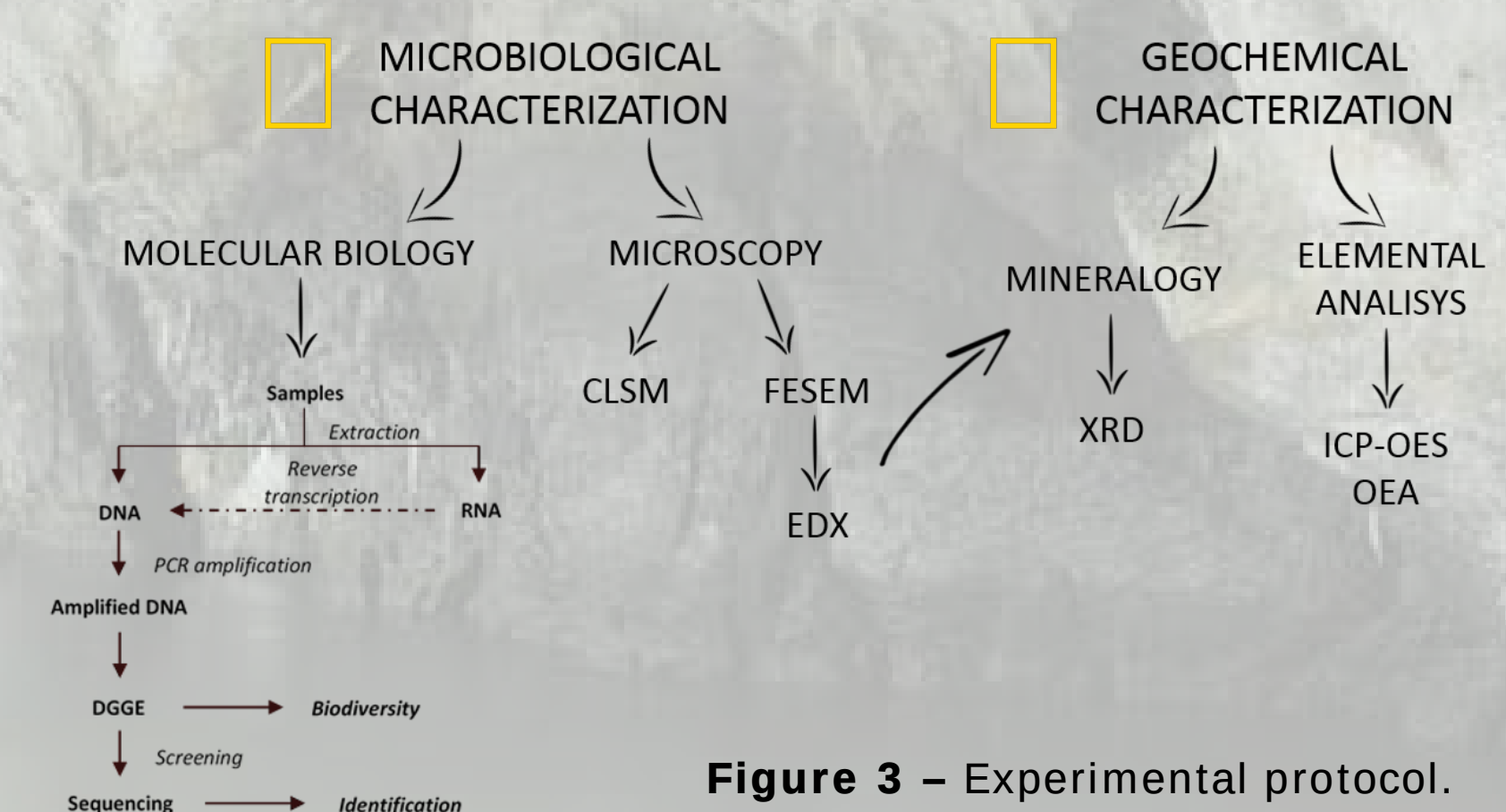


**Figure 3** – Experimental protocol.

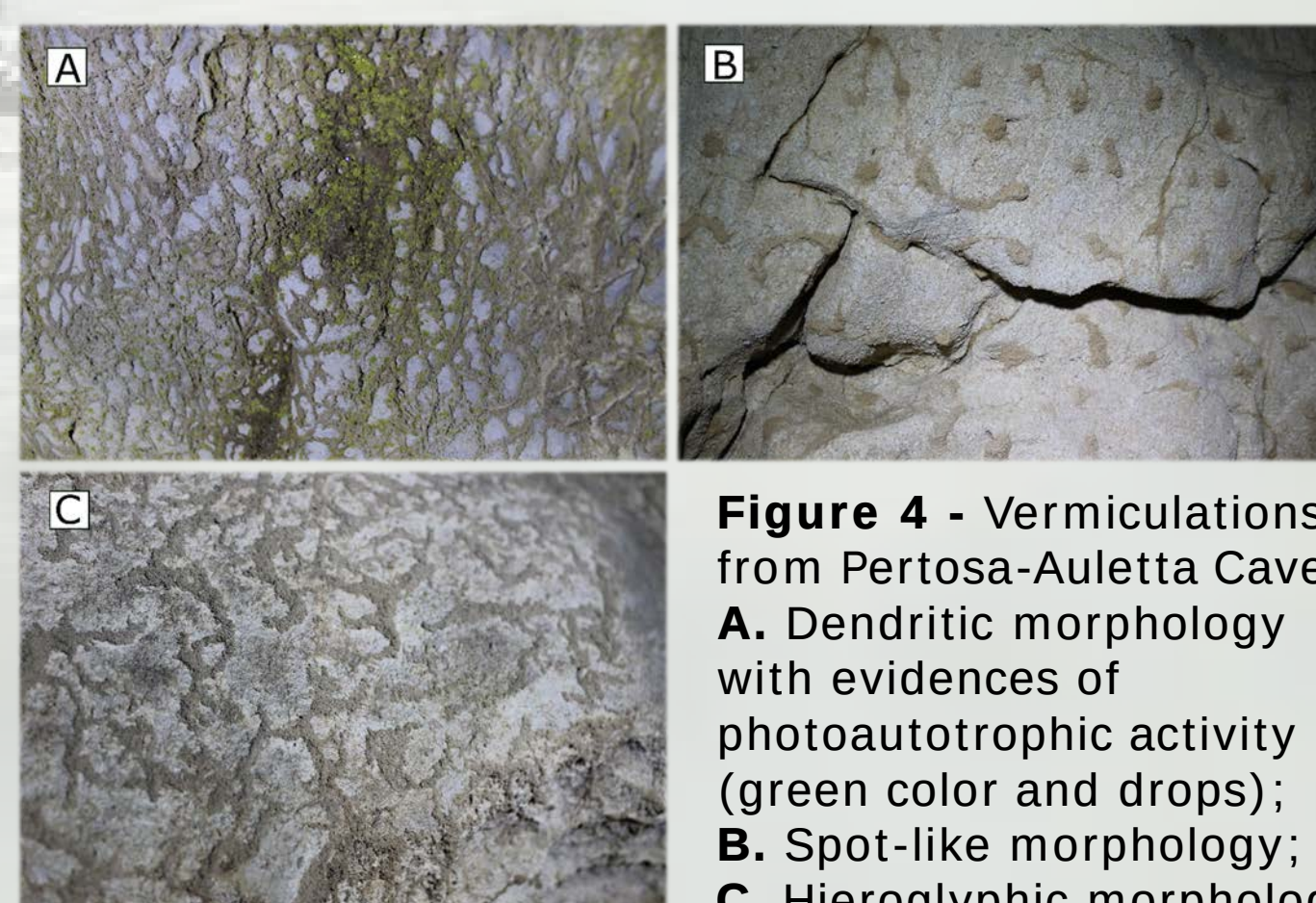


**Figure 4** - Vermiculations from Pertosa-Auletta Cave. A. Dendritic morphology with evidences of photoautotrophic activity (green color and drops); B. Spot-like morphology; C. Hieroglyphic morphology.

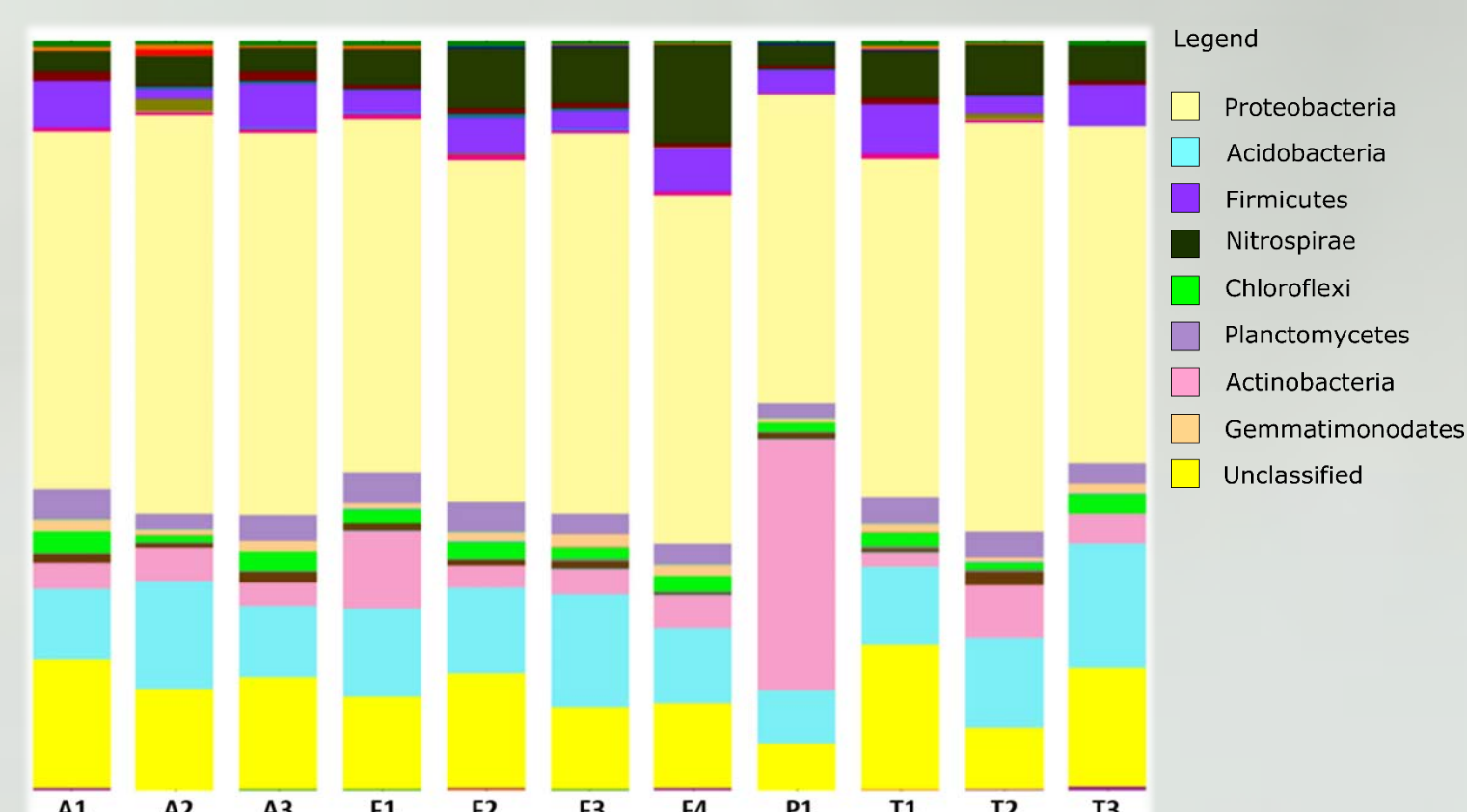


**Figure 5** - The main phyla for Bacteria in the analyzed samples.

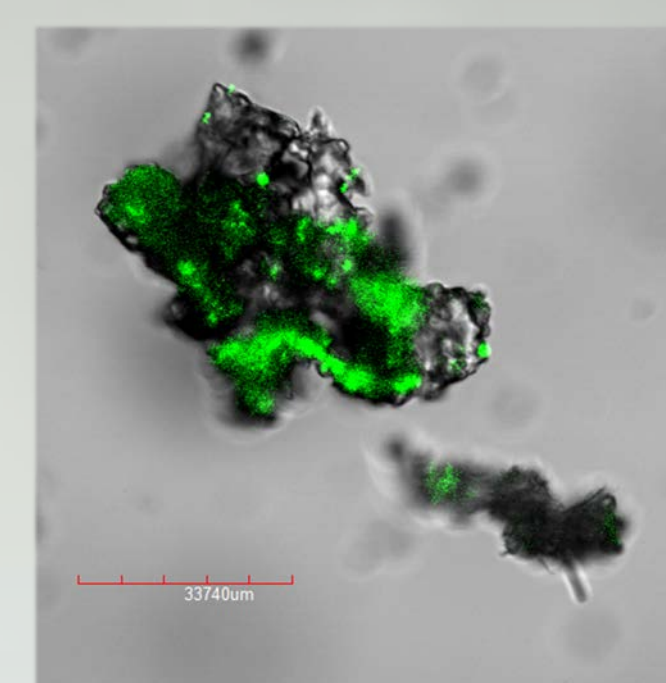


**Figure 6** – CLSM image showing that DNA exhibits a strange distribution and is clearly placed only in specific sites (deep green zones).

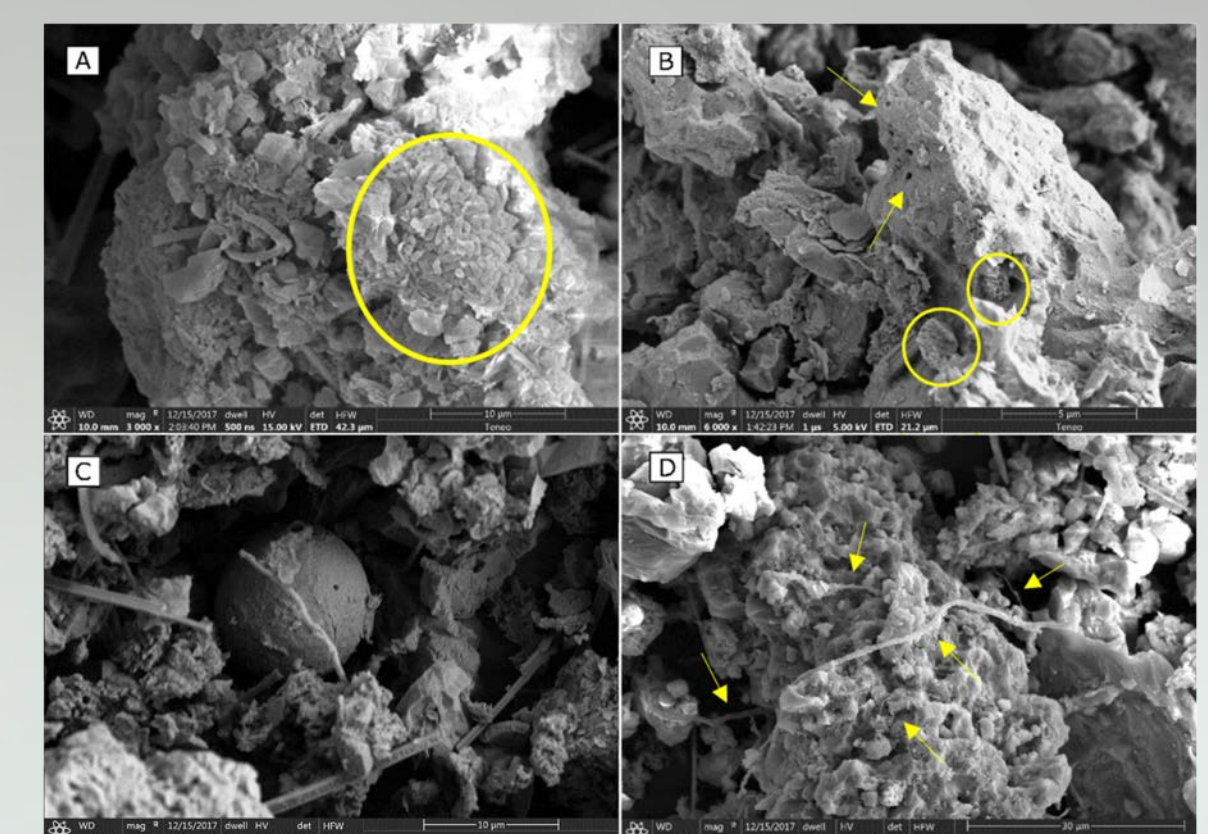


**Figure 7** - FESEM images of vermiculations. A. Clusters of cells; B. Etching voids typical of biological activity (see the arrows) and spores (see the rings); C. Calcite rods and vaterite minerals; D. Organic filaments.

#### Conclusions

This work supports the theory speculated by Jones et al. (2008), which suggests their genesis to be influenced by both physical and biological processes (6): the first one can determine the morphologies through wet-dry phenomena, as described by Bini et al. (1978) (3); whereas the microorganisms may play an active role in the formation and enrichment of vermiculation deposits, throughout a number of processes, like production of organic matter, precipitation of secondary minerals, enhancement of sediment trapping and binding, etching or pitting of the host rock.

#### References

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