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Biogenic calcium carbonate as evidence for life

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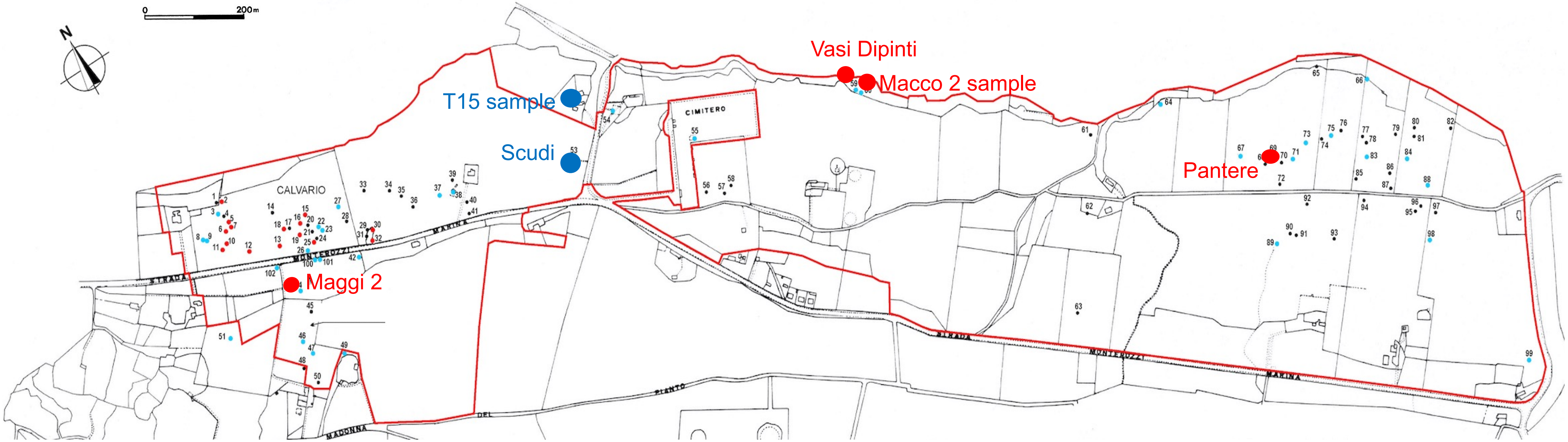
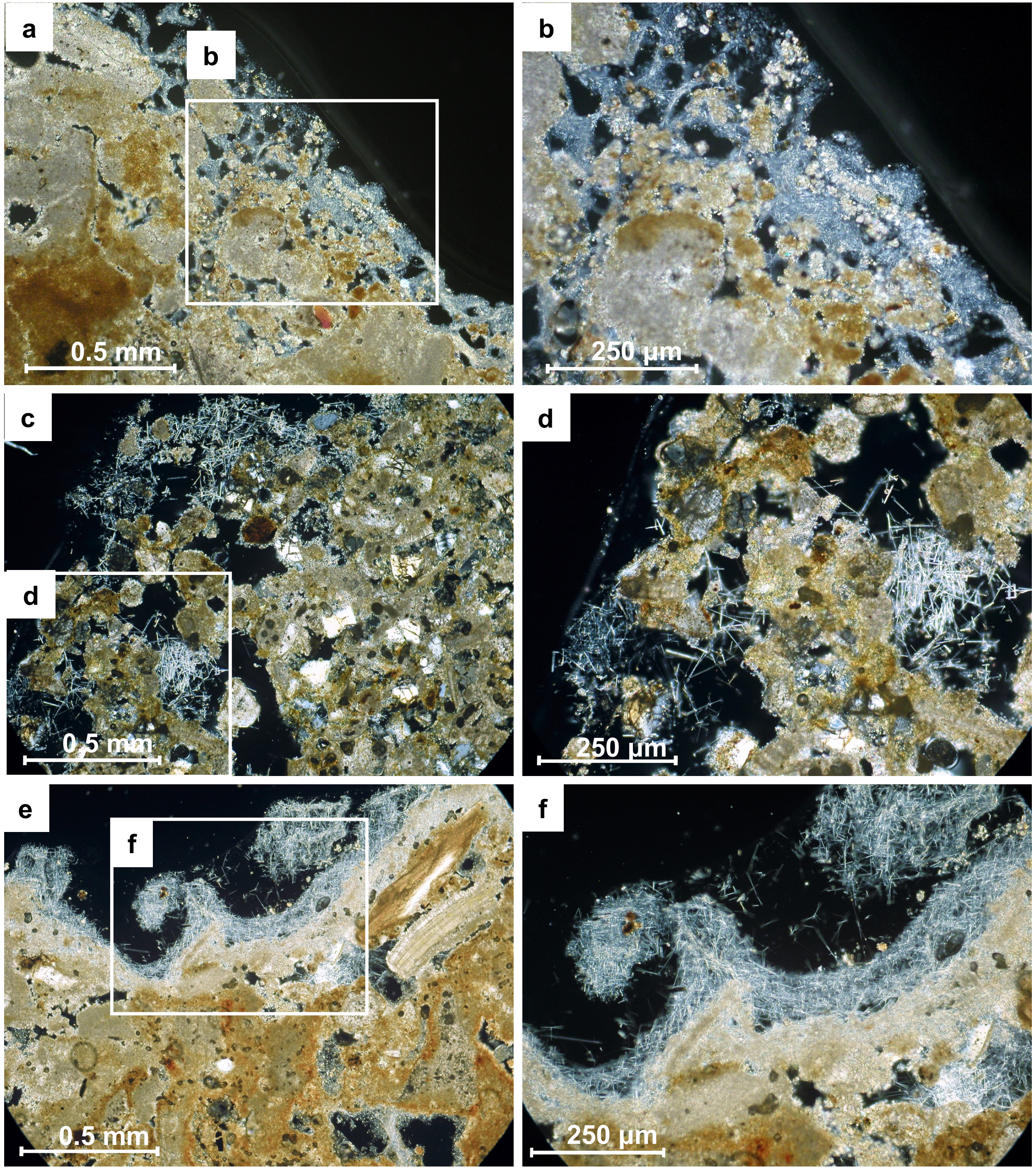
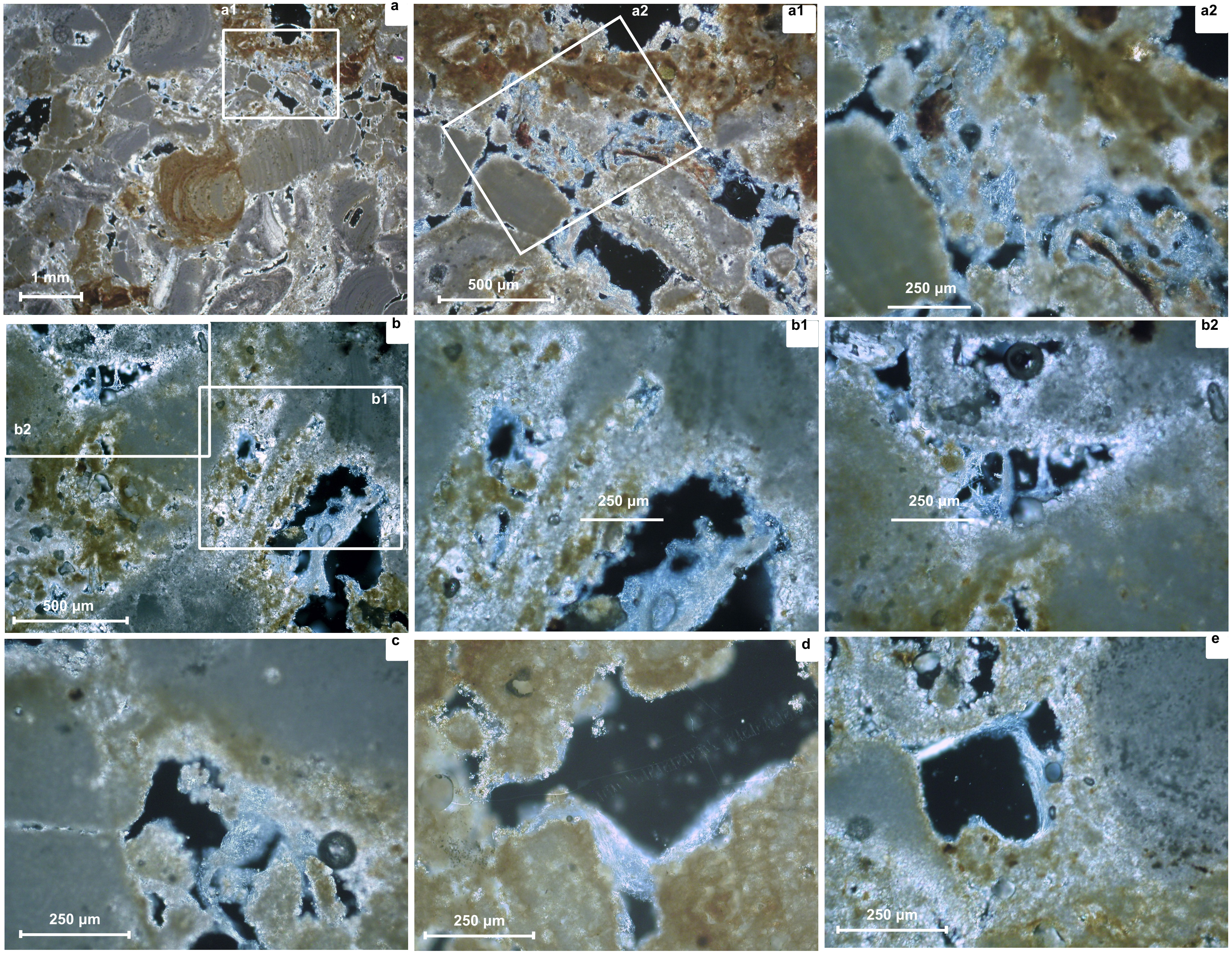


Name	Bedrock	Distance of the tomb ceiling below ground level (m)	Distance below the ground level where the samples were collected (m)	Thickness of moonmilk speleothem (cm)
Tomba delle Pantere	calcarenite	2	0	1.5 to 2
Tomba degli Scudi	hybrid sandstone	6	3	0.1 to 0.2
Tomba dei Vasi Dipinti	calcarenite	1,5	0	0,5
Tomba Maggi	calcarenite	3	4	1.5 to 2

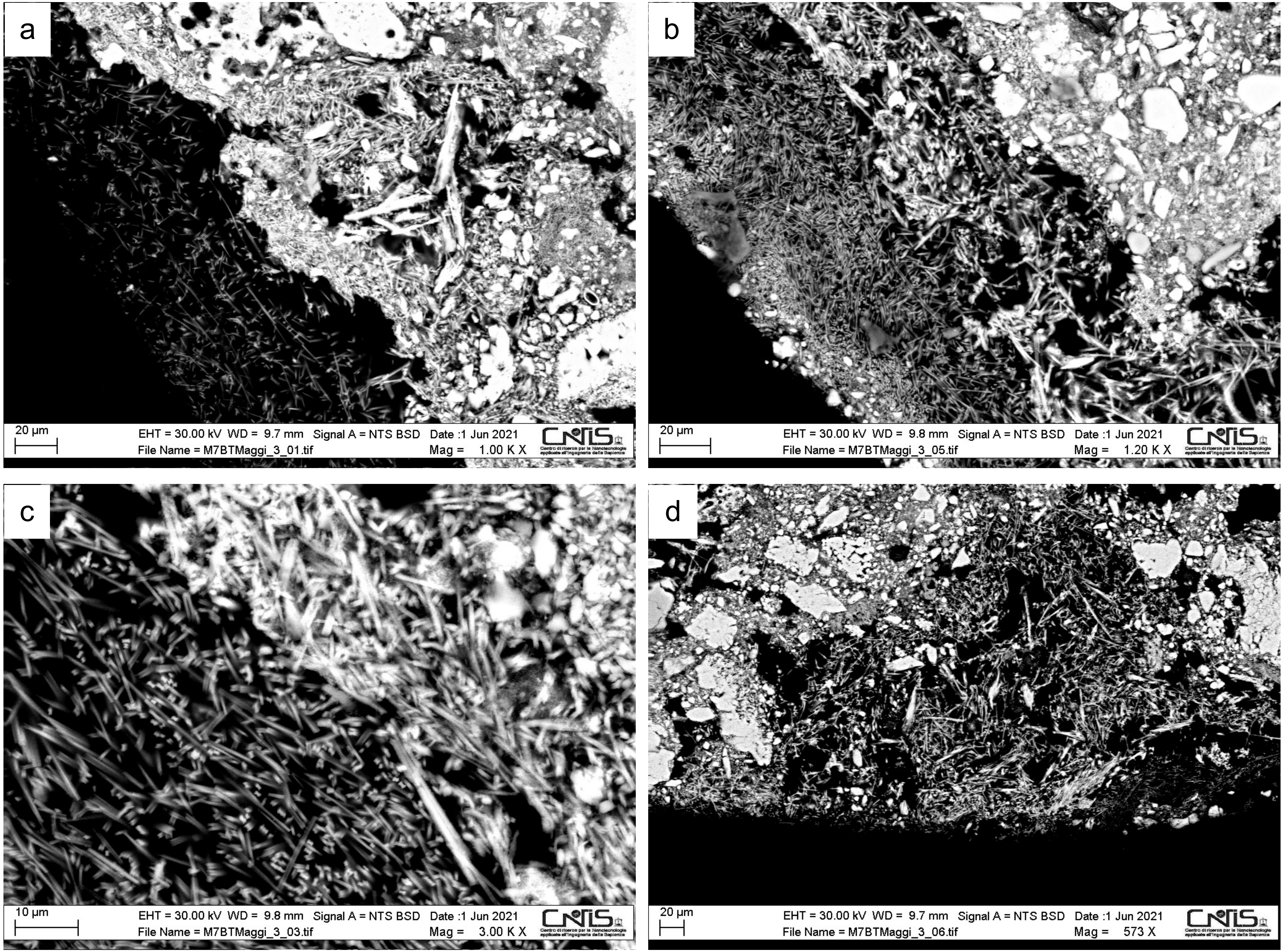
Supplement Fig. S1: map of the Necropolis of Tarquinia where the rocks and moonmilk samples were collected. Hybrid sandstone (blue dot) inside the *Tomba degli Scudi (42.247597, 11.777425),* calcarenite (red dots) outside the *Tomba delle Pantere* (42.241378, 11.791685), the *Tomba dei Vasi Dipinti* (42.246033, 11.784395) and inside the *Tomba Maggi 2* (42.248402,11.769660). The table indicates the distance of the tomb ceilings below the ground level, the distance below the ground level where the samples were collected, and the thickness of the moonmilk on the tomb walls. Hybrid sandstone sample T15 was collected from the substrate close to the *Tomba degli scudi*. Calcarenite sample Macco 2 was taken from the calcarenite substrate collected outside the *Tomba dei Vasi Dipinti.* Bar: 200 meters. The map was modified from Cirigliano et al., 2021a.



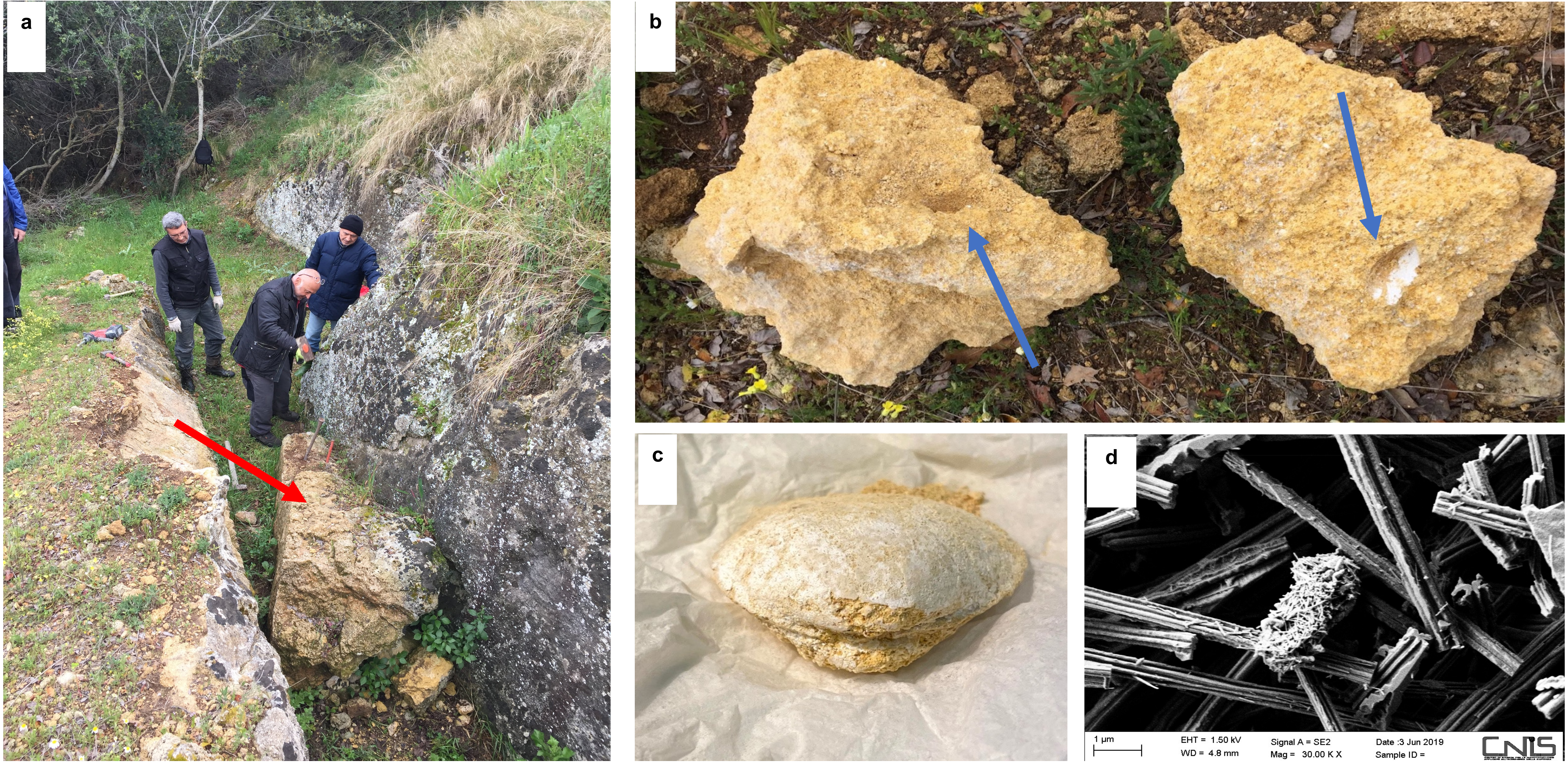
Supplement Fig. S2: the moonmilk ispresent on the surface and inside the calcarenite and the sandstone rocks. Optical microscope thin-section micrographs in crossed polarized transmitted light. **a,** calcarenite rock sampled outdoor of the *Tomba dei vasi dipinti*. **b**, enlarged view of the inset in **a**. **c**, hybrid sandstone sample collected inside the *Tomba degli Scudi*. **d**, enlarged view of the inset in **c.** **e,** a calcarenite sample collected inside the *Tomba Maggi 2*. **f,** enlarged view of the inset in e.



Supplement Fig. S3: the moonmilk contributes to the lithogenic processes. Optical microscope thin-section micrographs (crossed polarized transmitted light) of the moonmilk speleothems of calcarenite sampled from outdoor of *Tomba dei Vasi Dipinti*.

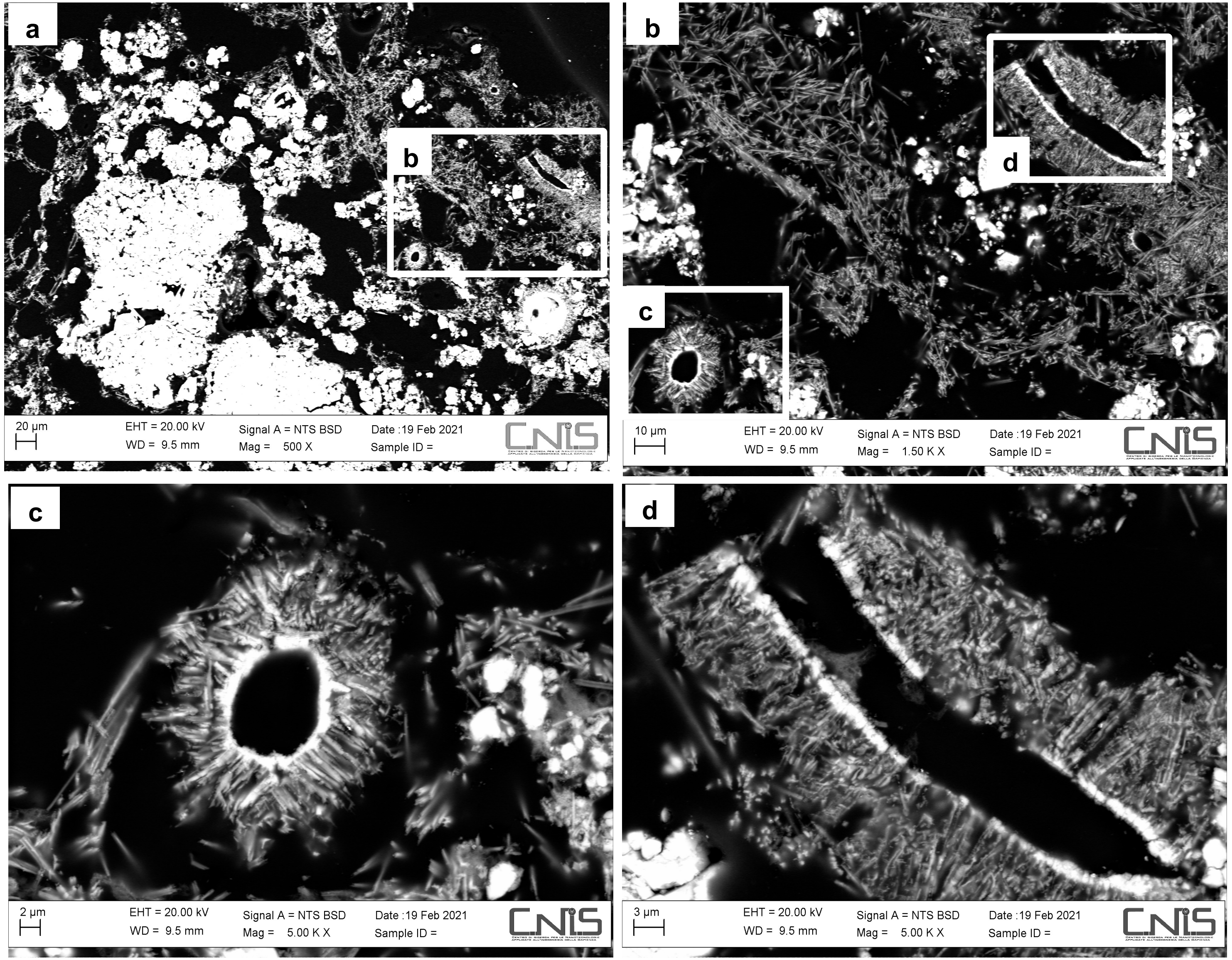


Supplement Fig. S4: the moonmilk contributes to the lithogenic processes. *S*canning electron micrographs of a thin-section of the calcarenite sampled indoor of *Tomba Maggi*.



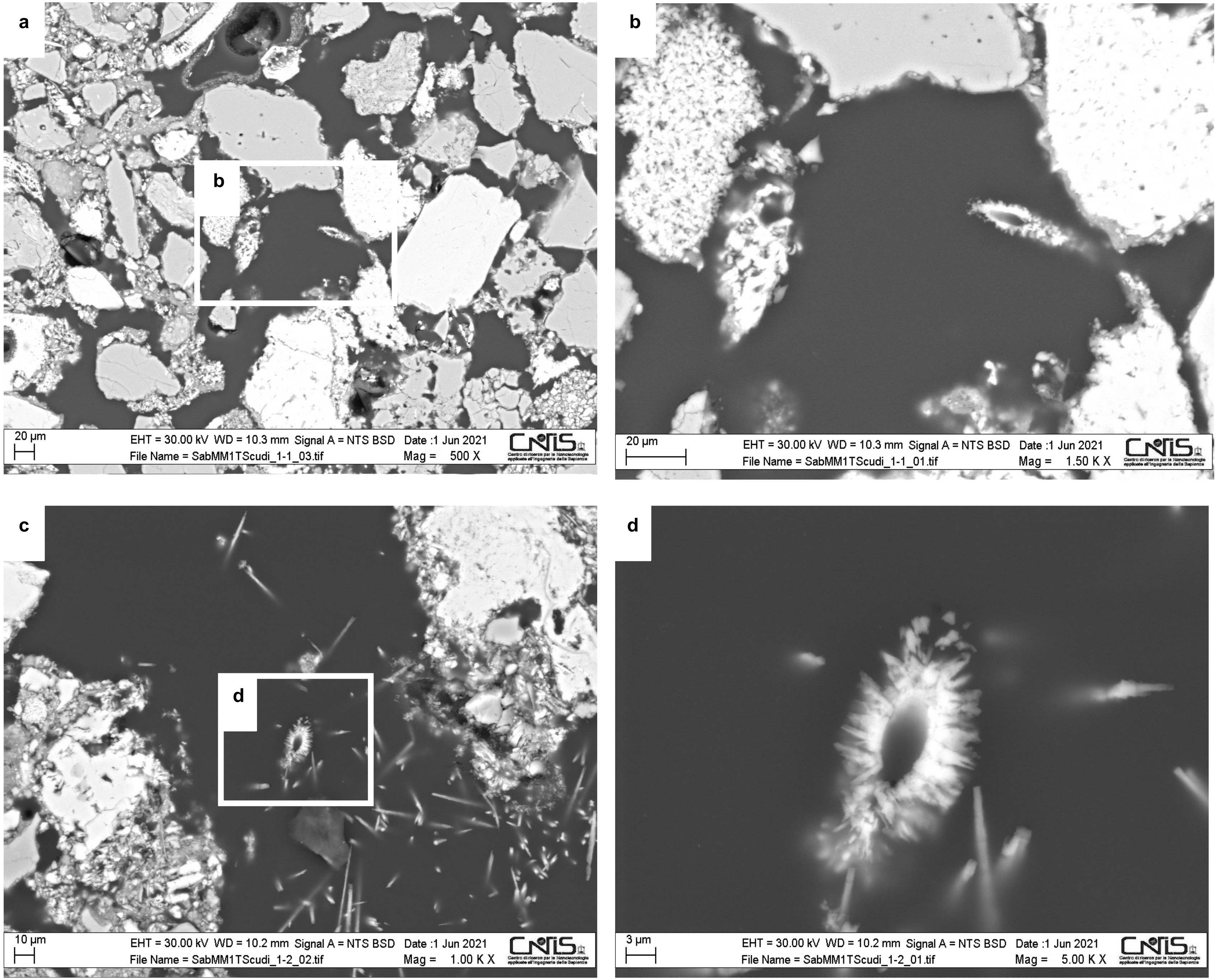
Supplement Fig S5. The moonmilk is naturally present deeply inside the calcarenite.

a, the entrance of the *Tomba dei Vasi Dipinti* was blocked by a rock slide; to remove the obstruction, the calcarenite rock (red arrow) was cut in pieces. **b**, the core of the rock contained a mould of a bivalve fossil (*Mactra* genus) covered by moonmilk on one side, blu arrows indicate the position of the fossil inside the rock. **c**, the mould of the bivalve fossil, length 4,5 cm and width 3 cm. **d**, Scanning electron micrographs of the moonmilk sampled from the rock shown in **b**. The moonmilk present inside the rock was more abundant in the interface between the rock and the shell of the bivalve, probably due to the higher space availability for the microbial community at the interface between the shell and the rock.

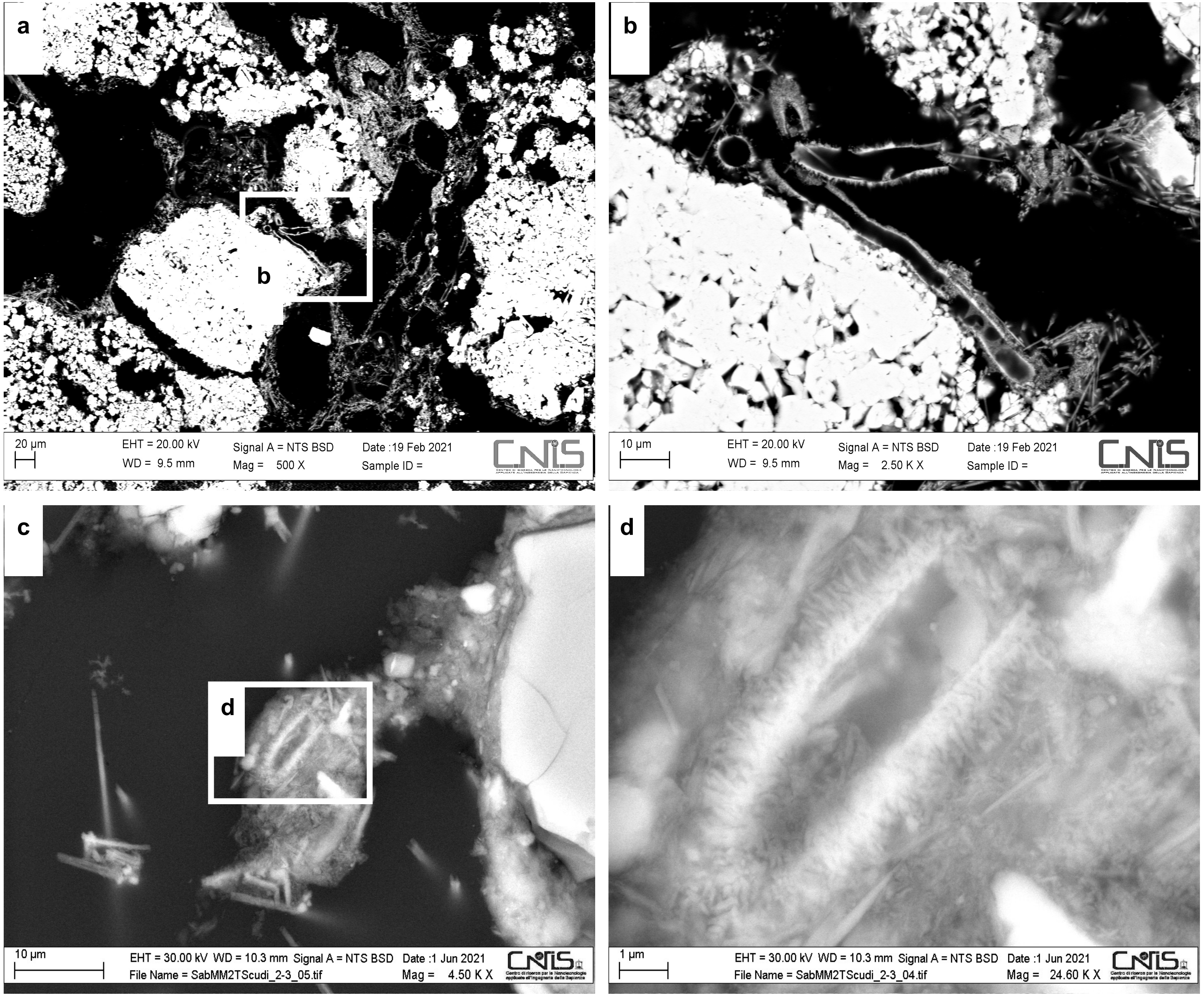


Supplement Fig. S6: bacterial biomineralization in the calcarenite sampled outdoor of the *Tomba dei Vasi Dipinti.*

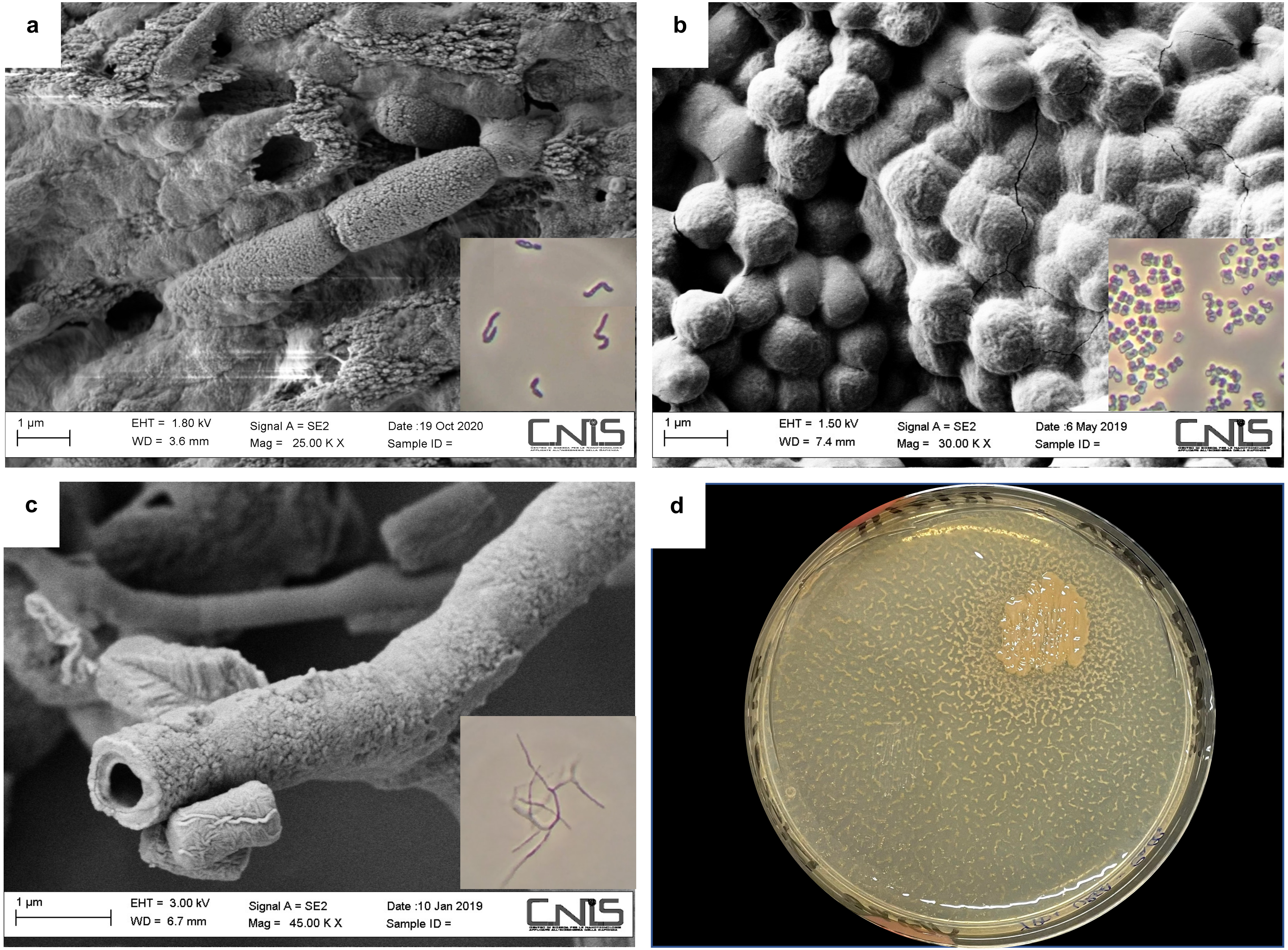
a, scanning electron micrographs of a thin-section. **b,** enlarged view of the inset in **a. c** and **d**: enlarged view of the inset in **b**.



Supplement Fig. S7: bacterial biomineralization in the hybrid sandstone sampled indoor the *Tomba degli Scudi*. Scanning electron micrographs of thin-sections (**a** and **c**). b, enlarged view of the inset in **a. d,** enlarged view of the inset in **c**.

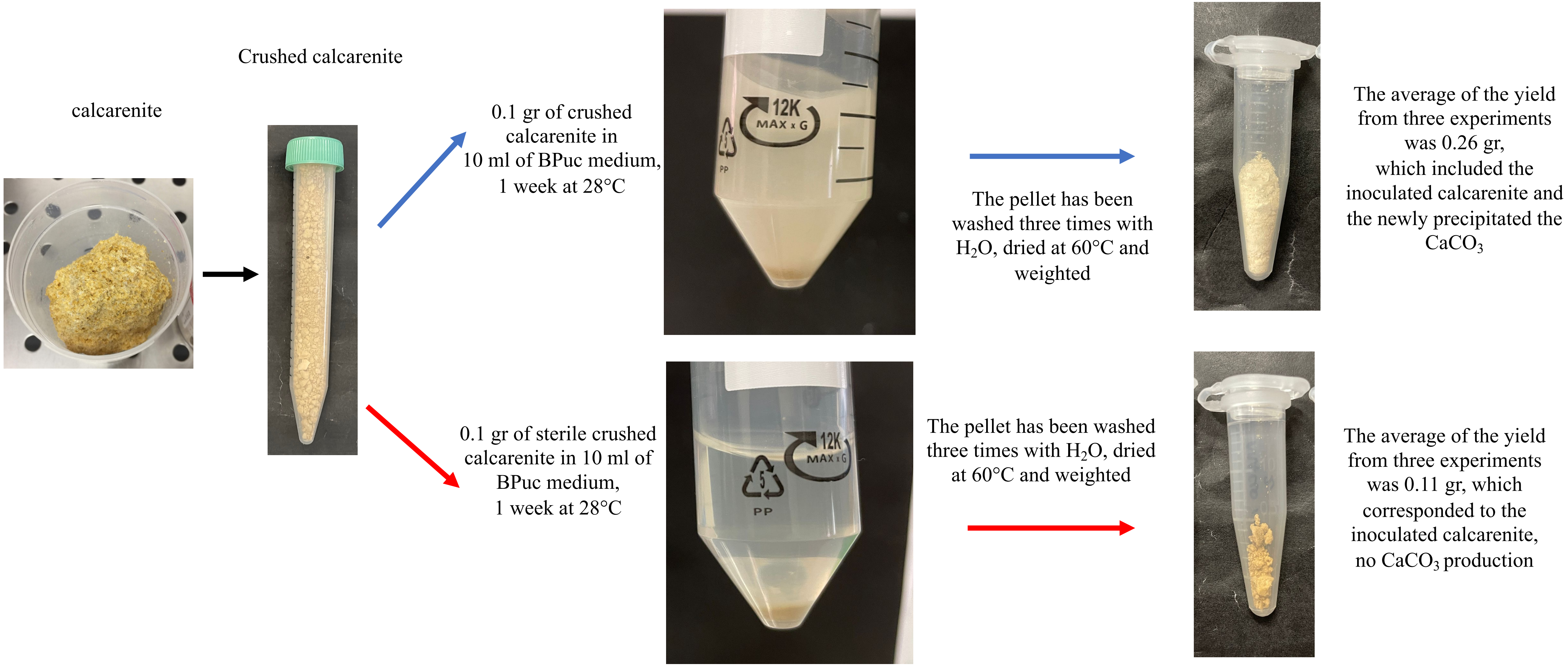


Supplement Fig. S8: bacterial biomineralization in the calcarenite.
a, *s*canning electron micrographs of a thin-section of the calcarenite sampled outdoor of the *Tomba delle Pantere* and **c** indoor of *Tomba Maggi 2*. **b** and **d**: enlarged view of the inset in **a** and **c**, respectively.

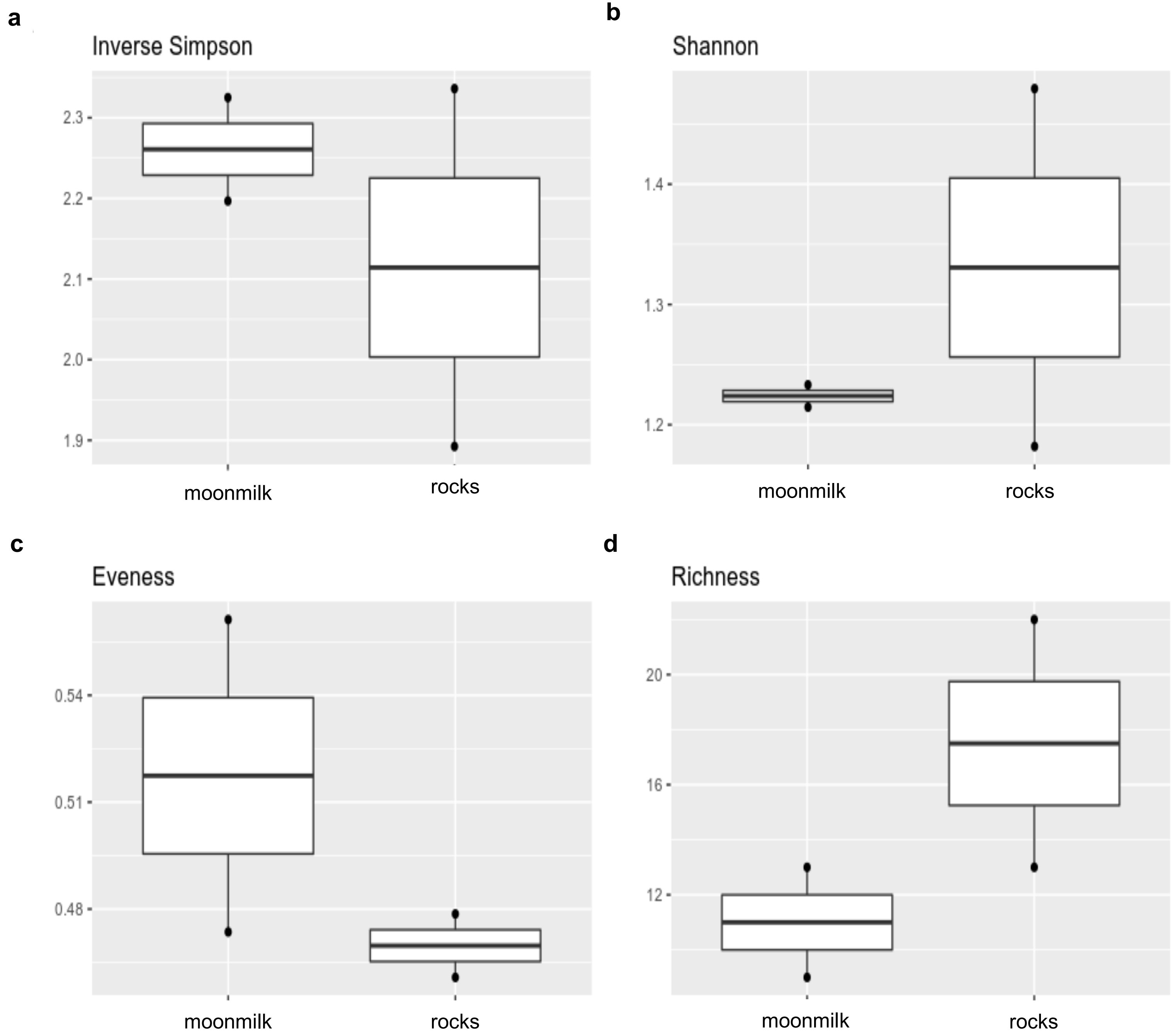


Supplement Fig. S9: examples ofbacterial biomineralization (entombment) in laboratory conditions.

Scanning electron micrographs of bacterial cultures grown in solid complete medium containing $CaCl_2$ and urea to induce calcium carbonate precipitation. **a,** *Bacillus*, **b,** *Sporosarcina* and **c,** *Streptomyces*. The insets correspond to optical microscope micrographs of the same bacterial strains (from our laboratory collection) grown in liquid LB medium. **d**, a carbonatogenic bacterial strain induces biomineralization extracellularly, in a plate (diameter 90 mm), even at distance from the cells. The strain (*Lysinibacillus fusiformis* 3.20 from our laboratory collection, Nigro et al., 2022) was grown in solid complete medium YPD containing $CaCl_2$ and urea, for one week at 28°C. This medium activates the ureolytic bacterial metabolism inducing calcium carbonate deposition, see Materials and Methods.



Supplement Fig. S10: the calcarenite harbours a microbial community that induces $CaCO_3$ precipitatation.
0.1 gr of crushed calcarenite was inoculated in BPuc medium (0.72% Bacto-peptone, 4% urea and 2.5% $CaCl_2$) and after one week at 28°C, the supernatant was discarded and the pellet was washed, dried and weighted (blue arrows). The same experiment has been performed with 0.1 gr of crushed calcarenite sterilized in an autoclave (red arrows). With the sterilized calcarenite no $CaCO_3$ production was observed, while the calcarenite rock containing a vital microbial community produced a considerable amount of $CaCO_3$, identified by microanalysis with a Scanning Electron Microscopy and X Ray Diffraction, not shown.



Supplement Fig. S11. Comparison of community alpha diversities between moonmilk and rocks.

Diversity was measured by inverse Simpson **a** and Shannon index **b**. The top and bottom boundaries of each box indicate the 75th and 25th quartile values, respectively. The black lines within each box represent the median values. There is any significant differences between the samples (Mann Whitney test, $P > 0.05$).

Evenness **c** and Richness **d** were measured by the Shannon Evenness index and the number of observed taxa, respectively. In moonmilk samples and rocks samples, the taxa richness was between 9 and 22 taxa (**b** and **d**). Consistently, evenness (Shannon evenness) and community richness (number of observed taxa) do not show any significant differences between the samples (Mann Whitney test, $P > 0.05$).