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Supplementary Material

Gypsum endolithic phototrophs under moderate climate (Southern Sicily): their diversity and pigment composition

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Table S1: Raman bands of the detected pigments.

Table S2: Table of samples used for metagenomics analysis, their lithology, characterization of endolithic colonization and relative abundance of endolithic phototrophs.

Figure S1: Micro-CT 3D reconstructions and selected slices illustrating the inner architecture and void space of gypsum fragments. Samples from Monte Perrera - MP1 (A – B) and MP2 (D – F); and from Monte Gibliscemi MG (G– I). The red and green frames in the 3D images show the position of the displayed slices.

Figure S1: Non-metric Multi-Dimensional Scaling (NMDS) analysis of the diversity among gypsum endolithic samples from two localities in Sicily. Samples from the studied localities are enclosed within convex hulls with centroids marked by asterisks (Siculiana Marina – black, full line, Santa Elisabetta – grey, dashed line). The analysis was based on Bray-Curtis distance matrix inferred from normalized abundances of the amplicon sequence variants (ASVs). The samples from Siculiana Marina exhibited greater overall diversity (they were also greater in number).

Figure S2: Non-metric Multi-Dimensional Scaling (NMDS) analysis of the diversity among gypsum endolithic samples from two different gypsum varieties. Samples from the studied substrates are enclosed within convex hulls with centroids marked by asterisks (selenite – black, full line, crystalline gypsum – grey, dashed line). The analysis was based on Bray-Curtis distance matrix inferred from normalized abundances of the amplicon sequence variants (ASVs). The samples from selenite exhibited greater overall diversity (but they were also significantly greater in number).

Figure S3: Non-metric Multi-Dimensional Scaling (NMDS) analysis of the diversity among gypsum endolithic samples from two differently coloured layers of colonization. Samples from the studied

endolithic layers are enclosed within convex hulls with centroids marked by asterisks (black zone – black, full line, green zone – grey, dashed line). The analysis was based on Bray-Curtis distance matrix inferred from normalized abundances of the amplicon sequence variants (ASVs). The samples from black layers exhibited greater overall diversity.

Site	Sample/ colour of colonisation	Organism	Identified pigment	Detected Raman bands
Siculiana Marina	1CF black	*Nostoc* sp.	scytonemin	1712 w, 1633 m, **1600 s**, 1558 m, 1387 w, 1324 w, **1174 ms**, 1098 mw, 1023 w, 755 w, 681 w
		Gloeocapsa sp.	gloeocapsin	1671 m, 1289 m, 480 mw
		Gloeocapsopsis pleurocapsoides	carotenoid	**1516 vs**, 1449 w, 1388 w, 1283 mw, 1189 mw, **1155 ms**, 1004 m, 957 w
Monte Perrera site	S12 black	*Gloeocapsa compacta*	gloeocapsin	**1668 s**, **1571 s**, **1424 ms**, 1340 m, **1280 ms**, 1190 m, 465 m, br
		Nostoc sp.	scytonemin	1711 w, 1633 m, **1598 s**, 1555 m, 1381 mw, **1171 ms**, 573 w, 436 w
		Nostoc sp.	carotenoid	**1513 vs**, 1283 w, 1193 m, **1155 s**, 1003 m, 958 w
Monte Gibliscemi	S14 orange	*Chlorophyta*	carotenoid	**1517 s**, 1447 w, 1389 w, 1274 w, **1191 ms**, **1156 s**, 1005 m, 964 w

Table S1: Raman bands of the detected pigments.

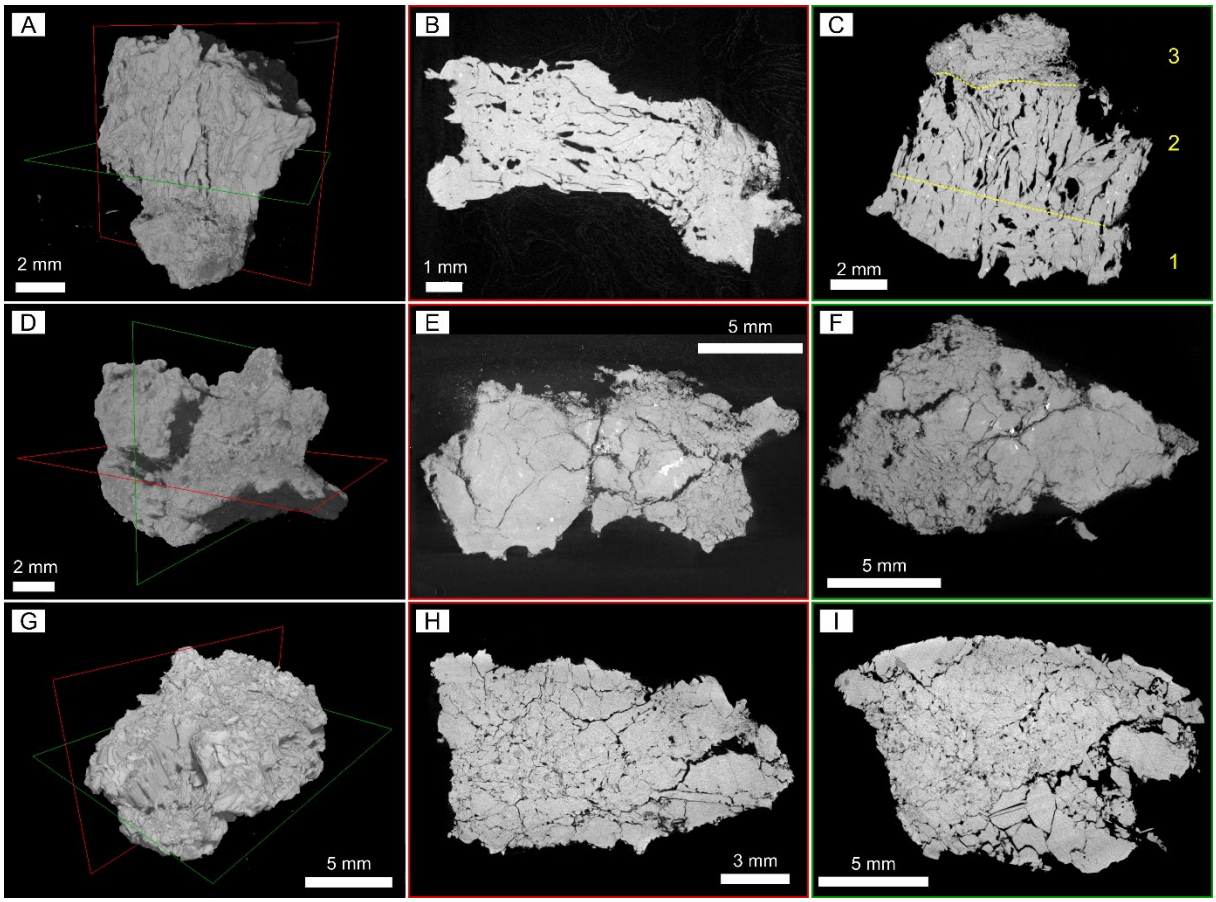
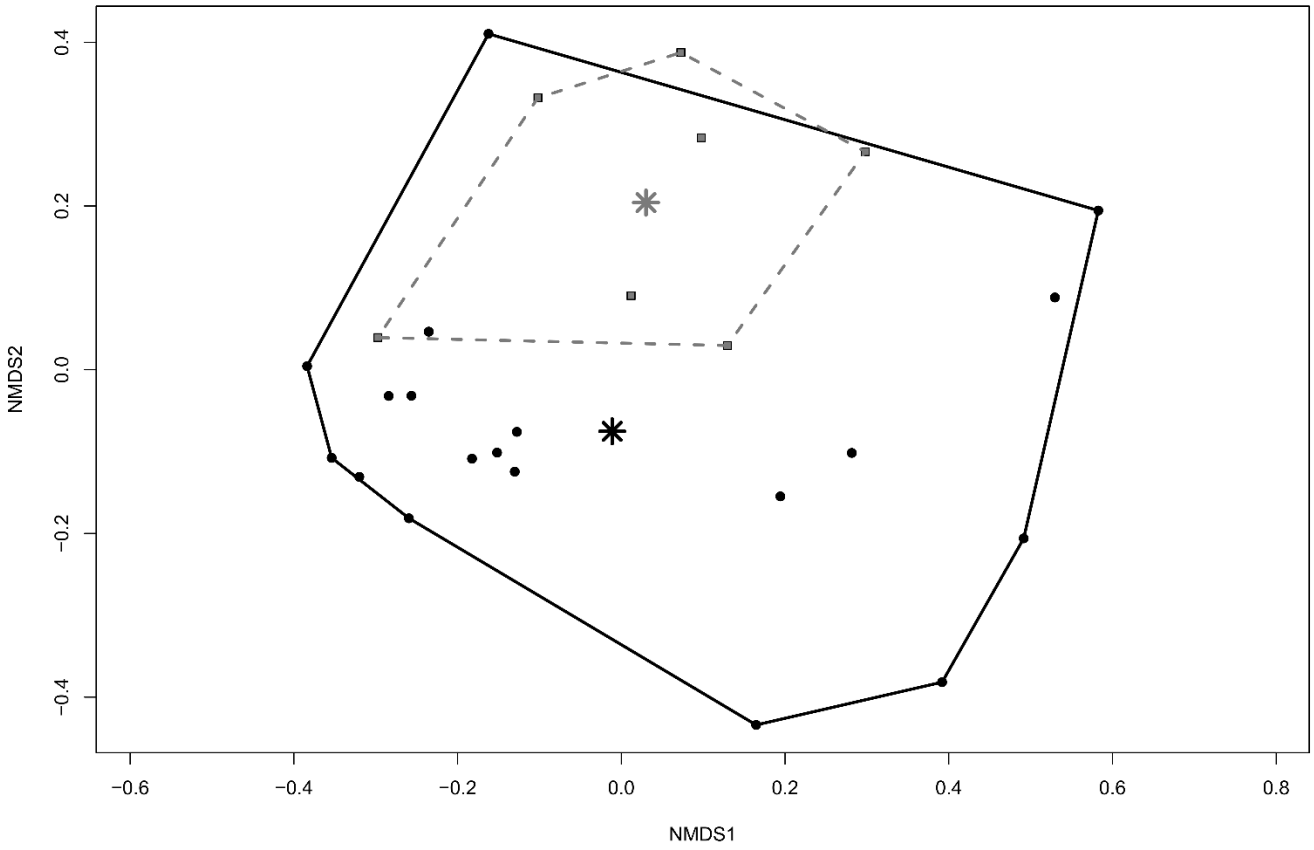


Figure S1: Micro-CT 3D reconstructions and selected slices illustrating the inner architecture and void space of gypsum fragments. Samples from Monte Perrera - MP1 (A – B) and MP2 (D – F); and from Monte Gibliscemi MG (G– I). The red and green frames in the 3D images show the position of the displayed slices.

Site	Lithology	Layer	Sample ID	Relative abundance		
				< 5 %	5-49 %	> 50 %
Siculiana Marina	Selenite	Green	1	*Nostoc* sp., *Microcoleus* sp., *Anathece* sp.	*Chroococcidiopsis* sp., *Chroococus* sp., *Gloeocapsa* sp., *orange-pigmented algae*	-
			2	*Gloeocapsa novacekii, G.rupestris, Nostoc* sp., *Chroococcus* sp.	*Chroococcidiopsis* sp., *Gloeocapsa* sp.	-
			3	*Nostoc* sp. *G. rupestris*	*Gloeocapsa* sp.	*Chroococcidiopsis* sp.,
			4	*Chroococcus, G. Compacta, Nostoc* sp., *Anathece* sp., *Petalonema* sp.	*Gloeocapsa* sp., *G.rupestris*	*Chroococcidiopsis* sp.,
			5		*Chroococcidiopsis* sp., *G. rupestris, G. novacekii*	*Gloeocapsa* sp.
			6	*Chroococcus* sp., *G. compacta*	*Chroococcidiopsis, Gloeocapsa* sp., algae	-
		Black	7		*Chroococcidiopsis* sp., algae	*Nostoc* sp.
			8	*Gloeobacter violaceus, Nostoc* sp., *Chroococcidiopsis* sp., *G. novacekii*	, *Gloeocapsa* sp., *G. rupestris,* algae	*G. compacta*
			9			*Nostoc* sp.
			10	algae		
			11	*Chroococcidiopsis* sp.	*Nostoc* sp., *Gloeocapsa* sp.	*Petalonema* sp.
			12	*Petalonema* sp., algae	*Gloeocapsa* sp.	*Nostoc* sp.
			13	*G. novacekii, Nostoc*	*G. pleurocapsoides, G. rupestris, Gleocapsa* sp. *(bezbarvá)*	*G. compacta*
	White crystalline	Green	14	*Gloeocapsa* sp., *G.compacta, Nostoc* sp.	-	*Chroococcidiopsis* sp.,
		Black	15	*Petalonema* sp., *Chroococcidiopsis* sp., *Nostoc* sp.	*Gloeocapsa* sp., *G.compacta, G. biformis, G. rupestris, Gloeobacter violaceous*	-
		Green	16	*Gloeocapsa* sp., *Nostoc*		*Chroococcidiopsis* sp.,
		Black	17	*Gloeocapsa* sp., *G. novacekii, Nostoc* sp.	*Chroococcidiopsis* sp., *Gloeobacter violaceus, G. rupestris, G. compacta, G. biformis, Petalonema* sp.	-
		Green	18		*Nostoc* sp.	*Chroococcidiopsis* sp.,
		Black	19	*Petalonema* sp., *Chroococcus* sp., *G. novacekii,*	*Chroococcidiopsis* sp., *Nostoc* sp., *G. compacta*	-
		Black	20	*Nostoc* sp., *G. novacekii*	*Gloeocapsa* sp., *G. compacta, G. rupestris, Chroococcidiopsis* sp.	-
Santa Elisabetta	Selenite	green	21	*Petalonema* sp., algae	*Gloeocapsa* sp., algae	*Chroococcidiopsis* sp.,
		black	22		*Gloeocapsa* sp., *Nostoc* sp., *G. biformis,* algae	-
		black	23	*Chroococcidiopsis* sp., algae	*G. rupestris*	*Gloeobacter violaceus*
		black	24	Orange-pigmented algae	*Nostoc* sp.	*Chroococcidiopsis* sp.,
		green	25		*Chroococcidiopsis* sp., *Nostoc* sp.	*Gloeocapsa* sp.
		green	26	*Chroococcidiopsis* sp., algae	-	*Gloeobacter violaceus*
		black	27		*Nostoc* sp., *Chroococcidiopsis* sp., algae	-

Table S2: Table of samples used for metagenomics analysis, their lithology, characterization of endolithic colonization and relative abundance of endolithic phototrophs.



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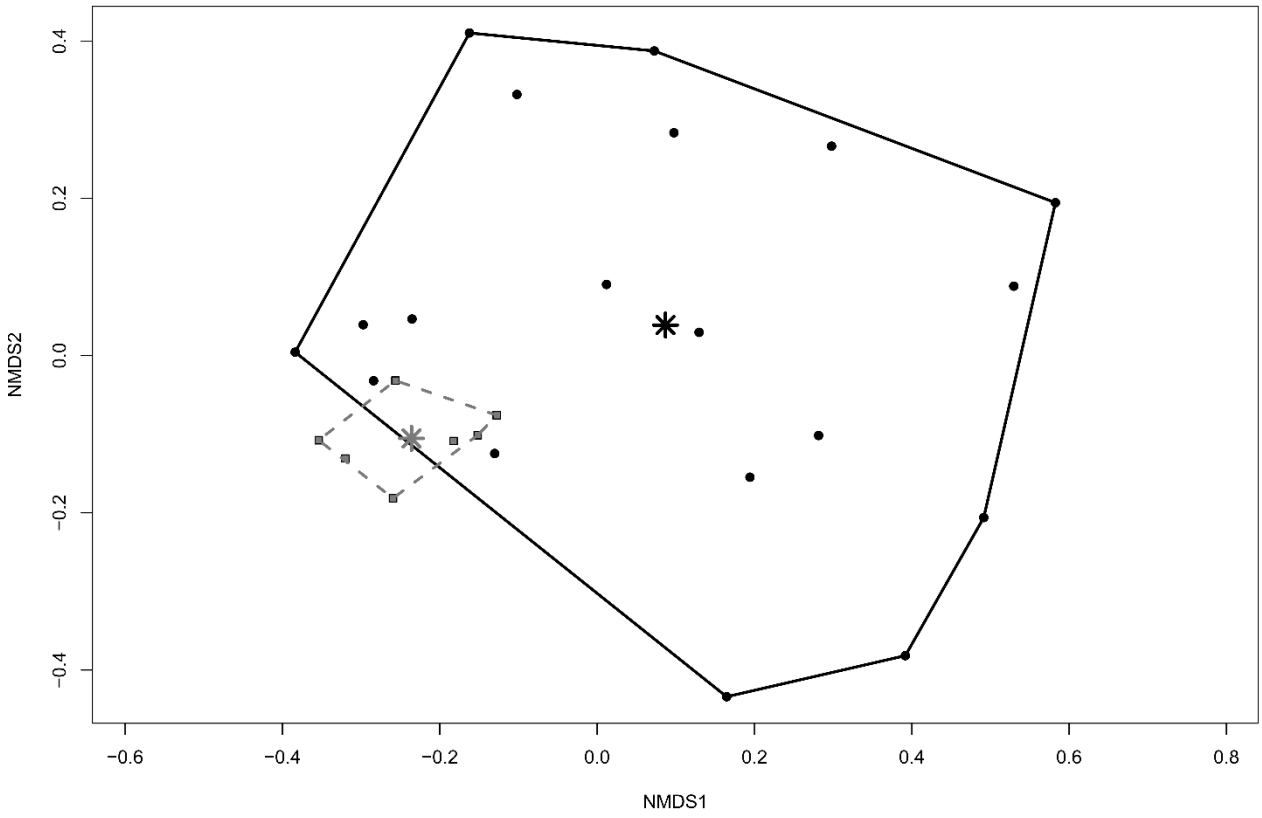

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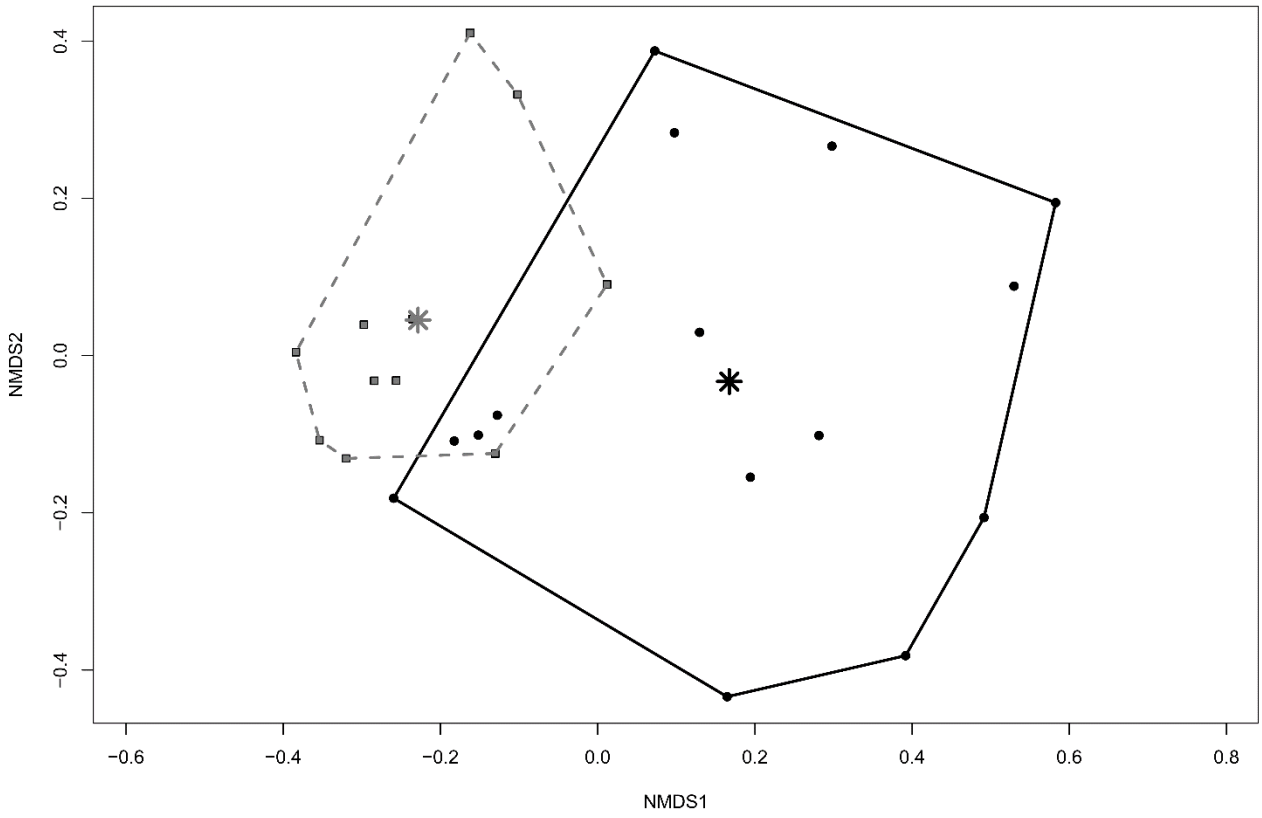

Figure S4: Non-metric Multi-Dimensional Scaling (NMDS) analysis of the diversity among gypsum endolithic samples from two differently coloured layers of colonization. Samples from the studied endolithic layers are enclosed within convex hulls with centroids marked by asterisks (black zone – black, full line, green zone – grey, dashed line). The analysis was based on Bray-Curtis distance matrix inferred from normalized abundances of the amplicon sequence variants (ASVs). The samples from black layers exhibited greater overall diversity.

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Accession numbers

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Cyanobacteria

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Algae

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