Tiny, glassy and rapidly trapped: the nano-sized planktic diatoms in the Messinian (late Miocene) gypsum

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This document describes the sampled sections, the methods, and the interpretation of the Raman spectra.

SAMPLED SECTIONS

The studied gypsum crystals were taken from unweathered outcrop surfaces from three sections (Perales, Banengo and Monticino). Samples were collected from the lower two beds of the Primary Lower Gypsum unit (PLG) (**Fig. DR1**). This unit was deposited in marginal Mediterranean basins during the early stage (5.97-5.60 Ma; Roveri et al., 2014) of the Messinian salinity crisis (MSC) and shows a precession-paced lithological cyclicity evidenced by shale/gypsum couplets. Shales reflect more humid climate at precession maxima, whereas gypsum was deposited during more arid climate conditions at precession minima (Lugli et al., 2010). Gypsum comprises different lithofacies: a) the massive selenite consisting of dm-thick, vertically oriented twinned selenite crystals (arrow-head or swallow-tail crystals); crystals show larger sizes in the lower two beds (cycles PLG1 and PLG2); b) the banded selenite consisting of smaller, vertical crystal palisades (less than 10 cm-thick) that are separated by mm-thick carbonate or clay laminae; c) the branching selenite consisting of meter-sized cones composed of mm- to cm-thick crystals with their long axis

inclined or oriented horizontally; the cones appear asymmetrical, with one side (branch) more developed than the other.

Figure DR1. Synthetic logs of the studied sections. Arrowheads indicate the sampled gypsum beds. The Monticino section is modified from Marabini and Vai (1989).

Perales section: this section (37°05'49''N, 2°03'19''W), located in the central portion of the Sorbas basin (SE Spain), is the reference section for the pre-MSC and MSC stratigraphy of the Western Mediterranean basin (Krijgsman et al., 2001; Sierro et al., 2001; Manzi et al., 2013). The section includes the pre-MSC Abad marls, a marly and diatomaceous unit showing a precession-driven lithological cyclicity, and the sulfate evaporites of the Yesares Formation, that is equivalent to the PLG unit. In the Perales section, this unit includes the lower three PLG cycles (**Fig. DR1**), all

composed of dm-sized twinned selenite crystals. The first gypsum bed is highly discontinuous. The second and third beds are laterally continuous. A total of 11 samples were taken from the Perales section, 5 from the first gypsum bed and 6 from the second gypsum bed.

Banengo section: this section (45°04'18''N, 8°03'24''E) is located in the Piedmont Basin (Northern Italy), at the northernmost offshoot of the Mediterranean. Here precession-paced pre-MSC shales and marls are overlain by the PLG unit, including four lithological cycles (Dela Pierre et al., 2015). The first and second gypsum beds (belonging to cycles PLG1 and PLG 2) were sampled (6 samples in total, 3 from the first bed and 3 from the second bed). These beds are composed of dm-sized twinned selenite crystals and are about 10 m thick.

Monticino section: the section (44°13'29''N, 11°45'43''E) is located in the Vena del Gesso (Northern Italy), a ridge exposing a complete succession of the PLG unit (Vai and Ricci Lucchi, 1977). Above pre-MSC shales, marls and carbonate-rich beds, seven PLG cycles are exposed, truncated at the top by an erosional unconformity in turn overlaid by upper Messinian post evaporitic clastic sediments (Marabini and Vai, 1989). A total of 4 samples were taken, 2 from the first and 2 from the second gypsum bed. These beds, about 5 m thick, and are composed of large (up to 2.5 m thick) selenite crystals (giant selenite; Lugli et al., 2010).

SAMPLE PREPARATION, OPTICAL MICROSCOPY AND SEM/EDS ANALYSES

Gypsum crystals were split along the cleavage plane using a rigid razor blade. In order to minimize possible contaminations, only the internal portion of the crystals has been subsampled, and the resulting fragments have been manipulated using latex gloves and tweezers. Petrographic thin sections from each crystal have been prepared, gluing representative polished subsamples onto glass slides (**Tab. DR1**); 47 smaller samples were not glued (**Tab. DR1**). Both thin sections and smaller samples were observed with a Leica DM 2770 P optical microscope equipped with a digital photcamera, at different magnifications (20X, 40X and 100 X). To prevent contamination, oil

immersion has been avoided throughout the use of dry lens objectives. The nano-sized planktic diatom aggregates have been documented at different focal planes, and the resulting photomicrographs have been stacked using the software ImageJ®. The fluorescence intensity of 6 unglued samples was evaluated using a Nikon microscope equipped with a B2-a filter block (illumination source with an excitation wavelength of 450-490 nm). SEM morphological observations and EDS qualitative analyses were performed on 11 partially dissolved and 9 completely dissolved samples prepared at Torino and Yamagata Universities. The former have been obtained by putting each sample in a small beaker and adding few ml of HCl 32%, leaving it to soak for about 5 hours; then the samples were carefully washed with pure water, placed into a Petri dish and left to dry in an oven at ~40°C. Completely dissolved samples have been obtained by placing each sample onto a nitrocellulose filter (47 mm diameter; 0.45 µm pore size) mounted onto a beaker supported by a filtration ramp linked to a vacuum pump; ~300 ml of pure water were added to the beaker, leaving it to soak for about 4 hours; then the water was pumped out and ~300 ml were added again; such procedure was repeated until the gypsum was completely dissolved. The filter was subsequently placed into a small, plastic Petri dish and left to dry for one night at room temperature. Then, a ~5x5 mm piece of the filter was carefully cut out and glued onto an aluminum stub. SEM morphological observations on sputter gold coated partially and completely dissolved samples were performed at Yamagata University using a JEOL JSM6510JLV SEM. SEM/EDS analyses on diatom valves were performed in low vacuum modality, on uncoated samples at Torino University, using a JSM IT300LV.

PERIODIC ACID SCHIFF COLORATION

Periodic Acid Schiff (PAS) staining, aimed at further confirming the presence of organic matter and, in particular, at verifying the potential occurrence of carbohydrates and/or glycoconjugates (Layton and Bancroft 2018), was performed on 1 unglued sample. Substances with

positive PAS reactions are colored in shades of red. The staining was carried out following the method of Whitlach and Johnson (1974). In particular, one gypsum sample was split under a stereomicroscope (Olympus SZH10) and two fragments (approximately $2 \times 1 \times 0.1$ mm) were stored in 70% ethyl alcohol for 40 minutes and then agitated with periodic acid for around 5 minutes. The samples were newly added with 70% ethyl alcohol, agitated again for 5 minutes, then washed with distilled water and agitated in Schiff's reagent for 10 minutes. Sodium metabisulfite was added and the samples were agitated for 3 minutes (such a step was repeated twice) and then washed with distilled water for 5 minutes (such a step was repeated twice). Finally, the samples were agitated in 70% ethyl alcohol for around 30 minutes, washed in deionized water and observed under a Nikon Eclipse 300 microscope. Most (but not all) of the nano-sized planktic diatoms were clearly PAS-positive, showing a discontinuous red-purple staining of the valve (**Fig. 3D**). The staining selectivity was likely related to the different position of the diatoms with respect to the sample surface and internal mechanical discontinuities. In this regard, both the short time agitation with periodic acid and HCl containing Schiff's reagent, likely favored the partial gypsum dissolution and thus the staining effectiveness.

RAMAN SPECTROSCOPY

Raman micro-spectroscopy was performed on 2 unglued samples with the Horiba JobinYvon LabRam HRVIS micro-/macro-Raman System of the Interdepartmental Center "G. Scansetti" at the Department of Earth Sciences (University of Torino). Excitation line at 532 nm (solid state Nd laser and 80 mW emission power) was used, with slit at 100 μ m and a grating of 600 grooves/mm. Each spectrum was collected in a confocal setting with a hole of 200 μ m and the laser was focused on the sample using an Olympus BX41 microscope with a 100x magnification (spot size resolution of ca. $1 \times 1 \times 3$ μ m). One to twenty accumulations in the time span of 1–15 s were

collected for each spectrum and D0.3 or D1 filters have been inserted at times. Calibration was performed using the 520.6 cm^{-1} Si band.

The organic matter has been analyzed on diatoms located a few microns below the surface of the gypsum crystal in order to minimize the signal of the hosting gypsum. The resulting spectra are characterized by fluorescence and by two broad bands centered at about 1350 and 1580 cm^{-1} . These bands correspond, respectively, to the D-band (disordered) and G-band (ordered band) of poorly organized carbonaceous material (Frezzotti et al., 2012), i.e. kerogen (**Fig. 3C**, spectrum 4). Moreover, the composition of diatom valves was characterized on one further sample that was dissolved onto a cover glass in order to exclude the very intense Raman signal of gypsum (**Fig. 3C**, spectrum 1). The obtained spectra (**Fig. 3C**, spectrum 3) were compared with a standard spectrum obtained from the valve of a large centric diatom isolated from the upper Miocene diatomaceous deposit of Piedmont Basin (Pellegrino et al., 2020; **Fig. 3C**, spectrum 2). Both the spectra are characterized by several large and intense overlapping bands between 300 and 500 cm^{-1} , by some weak overlapping bands around 800 cm^{-1} , and by a weak broad band at ca. 975 cm^{-1} . These bands correspond to those of the opal-A (Curtis et al., 2019).

Nr.	Sample name	Light microscopy	UV fluorescence	Raman spectroscopy	PAS coloration	SEM on partially dissolved sample	SEM on completely dissolved sample	Reference figure in the text
1	P1_1_1	X						Fig. 2G
2	P1_1_2	X	X					Fig. 2D-F; Fig. 3A-B
3	P1_1_3	X				X		Fig. 4E
4	P1_1_4	X				X		
5	P1_1_5	X				X		
6	P1_1_6	X		X				Fig. 3C
7	P1_1_7	X		X	X			Fig. 3D
8	P1_2_1	X						
9	P1_3_1	X						
10	P1_4_1	X						
11	P1_5_1	X						
12	P2_1_1	X						
13	P2_2_1	X						

14	P2_3_1	X						
15	P2_4_1	X						
16	P2_5_1	X						
17	P2_6_1	X						
18	P2_6_2	X	X				X	
19	P2_6_3	X						
20	P2_6_4	X						
21	P2_6_5	X						
22	P2_6_6	X						Fig. 4F
23	P2_6_7	X						
24	B1_1_1	X						
25	B1_2_1	X						
26	B1_2_2	X						
27	B1_2_3	X	X					Fig. 4C-D
28	B1_2_4	X						
29	B1_2_5	X					X	Fig. 2K-L
30	B1_2_6	X					X	Fig. 3E; Fig. 4B
31	B1_2_7	X				X		Fig. 2I
32	B1_2_8	X				X		
33	B1_2_9	X				X		
34	B1_2_10	X				X		
35	B1_3_1	X						
36	B1_3_2	X				X		
37	B1_3_3	X					X	
38	B1_3_4	X					X	
39	B1_3_5	X						Fig. 2A-C
40	B1_3_6	X					X	
41	B1_3_7	X					X	
42	B1_3_8	X				X		
43	B1_3_9	X				X		
44	B1_3_10	X				X		Fig. 2J; Fig. 4A
45	B2_1_1	X						
46	B2_1_2	X						
47	B2_1_3	X						Fig. 4H
48	B2_1_4	X	X					
49	B2_1_5	X					X	
50	B2_1_6	X					X	
51	B2_2_1	X						
52	B2_2_2	X						
53	B2_3_1	X						
54	B2_3_2	X						
55	M1_1_1	X						
56	M1_1_2	X						

57	M1_1_3	X	X					
58	M1_1_4	X						
59	M1_1_5	X						
60	M1_2_1	X						
61	M1_2_2	X						
62	M2_1_1	X						
63	M2_1_2	X						
64	M2_1_3	X						
65	M2_1_4	X	X					
66	M2_1_5	X						
67	M2_2_1	X						
68	M2_2_2	X						

Table DR1. List of subsamples analyzed and applied techniques (petrographic thin sections are reported in bold).

REFERENCES CITED

Curtis, N.J., Gascooke, J.R., Johnston, M.R. and Pring, A., 2019, A review of the classification of opal with reference to recent new localities: *Minerals* (MDPI), v. 9 (5), 299, 1-20.

Dela Pierre, F., Natalicchio, M., Ferrando, S., Giustetto, R., Birgel, D., Carnevale, G., Gier, S., Lozar, F., Marabello, D., and Peckmann, J., 2015, Are the large filamentous microfossils preserved in Messinian gypsum colorless sulfide-oxidizing bacteria?: *Geology*, v. 43 (10), p. 855-858, doi:10.1130/G37018.1.

Frezzotti, M.L., Tecce, F. and Casagli, A., 2012, Raman spectroscopy for fluid inclusion analysis: *Journal of Geochemical Exploration*, v. 112, p. 1-20, doi: 10.1016/j.gexplo.2011.09.009.

Krijgsman, W., Fortuin, A.R., Hilgen, F.J., Roep, T.B., and Sierro, F.J., 2001, Astrochronology for the Messinian Sorbas Basin (SE Spain) and orbital (precessional) forcing for evaporite cyclicity: *Sedimentary Geology* v. 140, p. 43–60, doi: 10.1016/S0037-0738(00)00171-8.

Layton, C., and Bancroft, J.D., 2018, Carbohydrates, *in*: Suvarna, S.K., Layton, C. and Bancroft, J.D., eds., Bancroft's theory and practice of histological techniques, 8th Edition, Amsterdam, Elsevier, p. 176-197.

Lugli, S., Manzi, V., Roveri, M., and Schreiber, B.C., 2010, The Primary Lower Gypsum in the Mediterranean: a new facies interpretation for the first stage of the Messinian salinity crisis: *Palaeogeography Palaeoclimatology Palaeoecology*, v. 297 (1), p. 83-99, doi:10.1016/j.palaeo.2010.07.017.

Manzi, V., Gennari, R., Hilgen, F., Krijgsman, W., Lugli, S., Roveri, M., and Sierro, F.J., 2013, Age refinement of the Messinian salinity crisis onset in the Mediterranean: *Terra Nova* v. 25, p. 315–322, doi:10.1111/ter.12038.

Marabini, S. and Vai, G.B., 1989, Geology of the Monticino quarry, Brisighella, Italy. Stratigraphic implications of its late Messinian mammal fauna: *Bollettino della Società Paleontologica Italiana*, v. 28, p. 369-382.

Pellegrino, L., Abe, K., Gennari, R., Lozar, F., Dela Pierre, F., Natalicchio, M., Mikami, Y., Jordan, R.W. and Carnevale, G., 2020, Integrated micropaleontological study of the Messinian diatomaceous deposits of the Monferrato Arc (Piedmont basin, NW Italy): New insights into the paleoceanographic evolution of the northernmost Mediterranean region: *Marine Micropaleontology*, v. 160 (101910), p. 1-19, doi:10.1016/j.marmicro.2020.101910.

Sierro, F.J., Hilgen, F.J., Krijgsman, W., and Flores, J.A., 2001, The Abad composite (SE Spain): a Messinian reference section for the Mediterranean and the APTS: *Palaeogeography Palaeoclimatology Palaeoecology*, v. 168, p. 141–169, doi:10.1016/S0031-0182(00)00253-4.

Vai, G.B., Ricci Lucchi, F., 1977, Algal crusts, autochthonous and clastic gypsum in a cannibalistic evaporite basin; a case history from the Messinian of Northern Apennine: *Sedimentology*, v. 24, p. 211–244, doi:10.1111/j.1365-3091.1977.tb00255.x.

Whitlatch, R.B. and Johnson, R.G., 1974, Methods for staining organic matter in marine sediments: *Journal of Sedimentary Petrology*, v. 44 (4), p. 1310-1312, doi 10.1306/212F6CAD-2B24-11D7-8648000102C1865D.