Marine and freshwater micropearls: biomineralization producing strontium-rich amorphous calcium carbonate inclusions is widespread in the genus *Tetraselmis* (Chlorophyta)

Supplementary Materials

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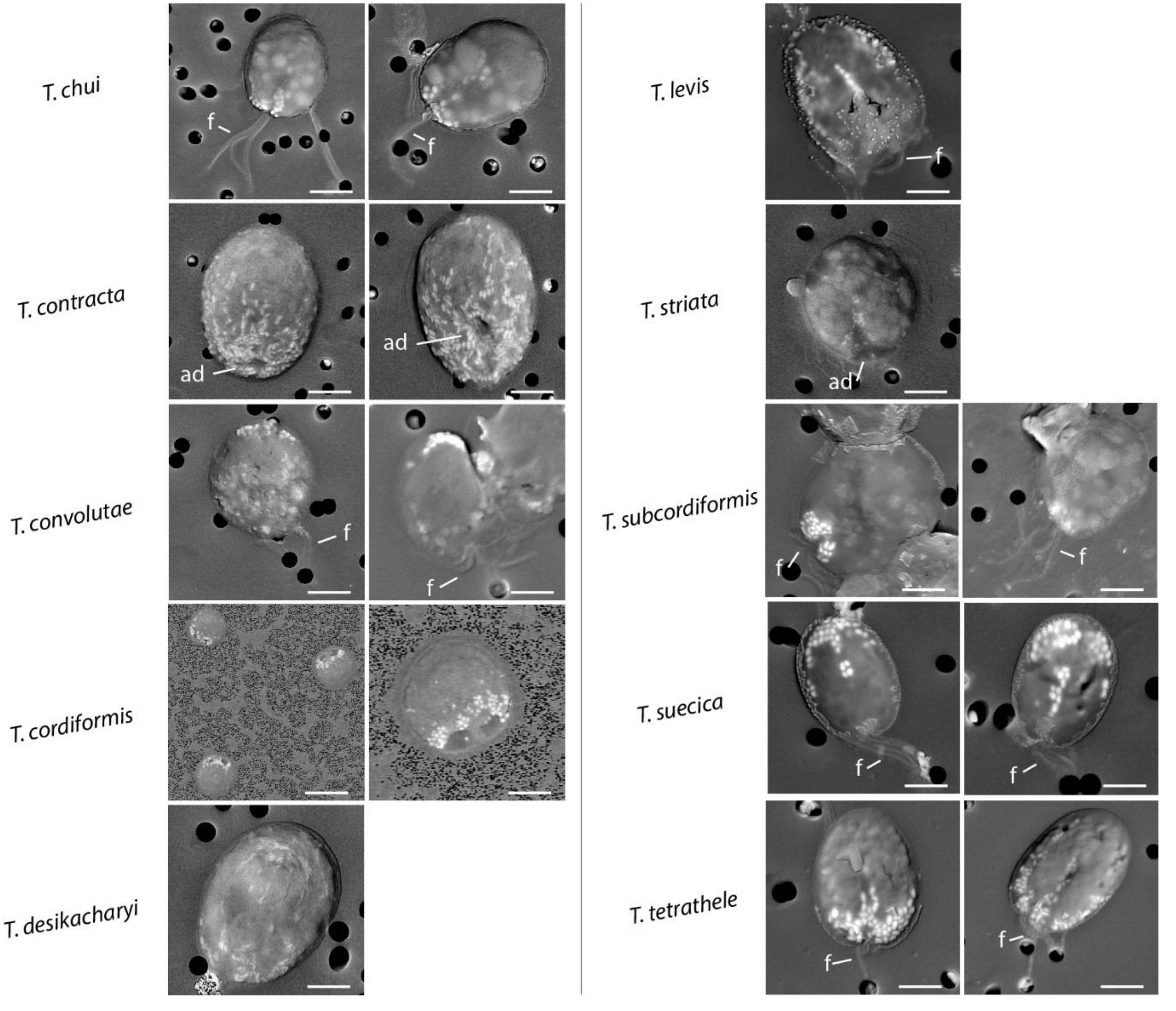


Figure S1: SEM images providing an overview of the micropearl location in *Tetraselmis* species.

Backscattered electron images of dried samples. The micropearls appear in white or light grey against the darker organic matter. The larger and slightly darker inclusions are polyphosphate (observed here in *T. chui*, *T. convolutae*, *T. striata*, *T. subcordiformis*). Pores of the filters are visible as black circles in the background (0.2/1 or 2 micrometers of diameter). The location of the micropearls is linked to the observation of flagella (f) or of the apical depression (ad). In *T. cordiformis*, the two contractile vacuoles are clearly visible and are located at the apical side of the cell. Finally, the orientation of *T. desikacharyi* stays completely uncertain, although similar observations in *T. convolutae* seem to indicate that the iron oxide minerals (in white) are formed around the (missing) flagella. Scale bars: 5μm.

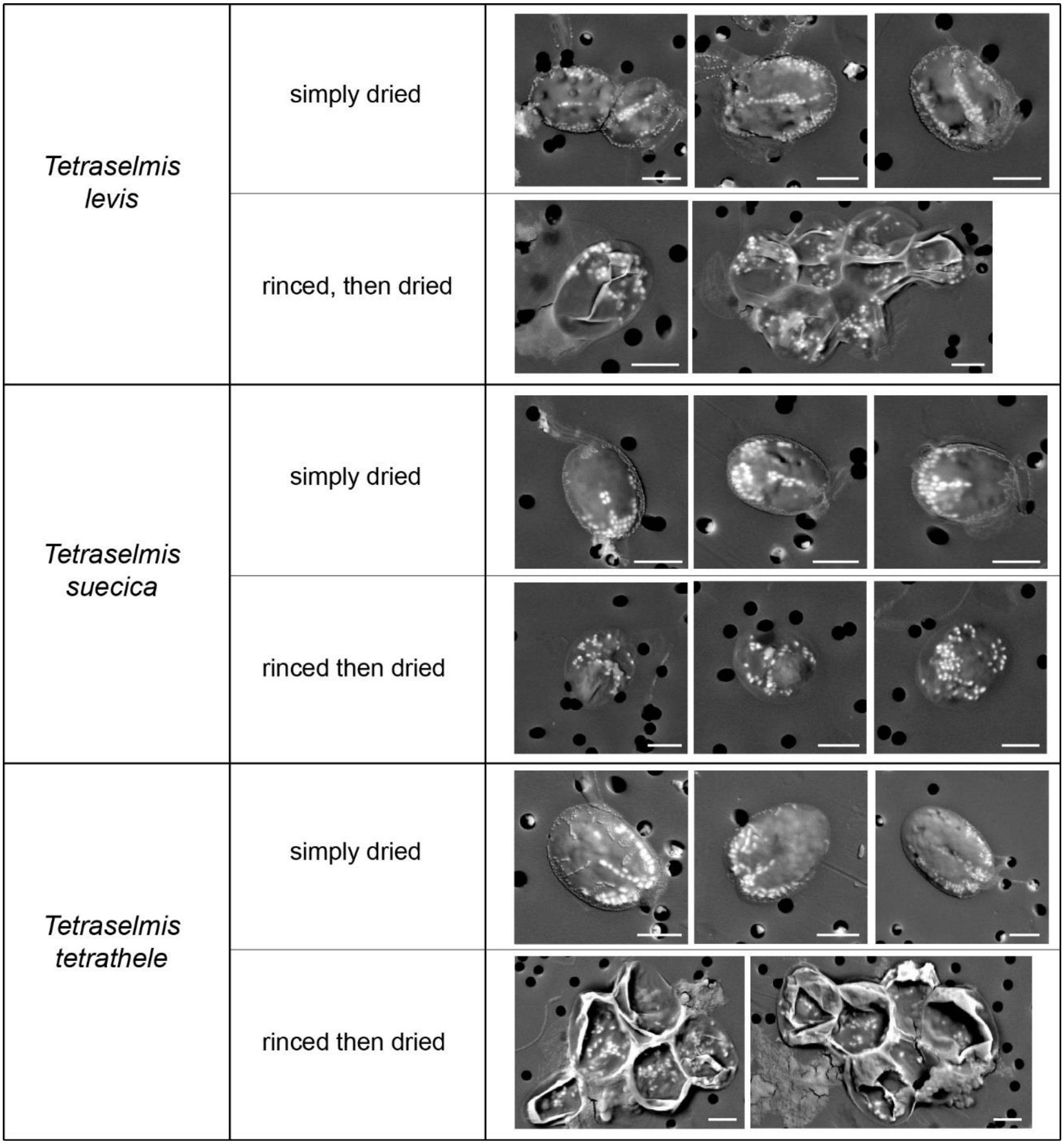


Figure S2: Micropearl distribution inside the cell disrupted by MilliQ water rinsing.

SEM backscattered electron images of dried samples. The micropearls appear in white or light grey against the darker organic matter. Each culture was sampled at the same time, but prepared in two different ways: either simply dried on a filter, either rinsed shortly with MilliQ water and then dried on a filter. The micropearls' distribution inside the cell is not preserved when the sample is rinsed. Scale bars: 5µm.

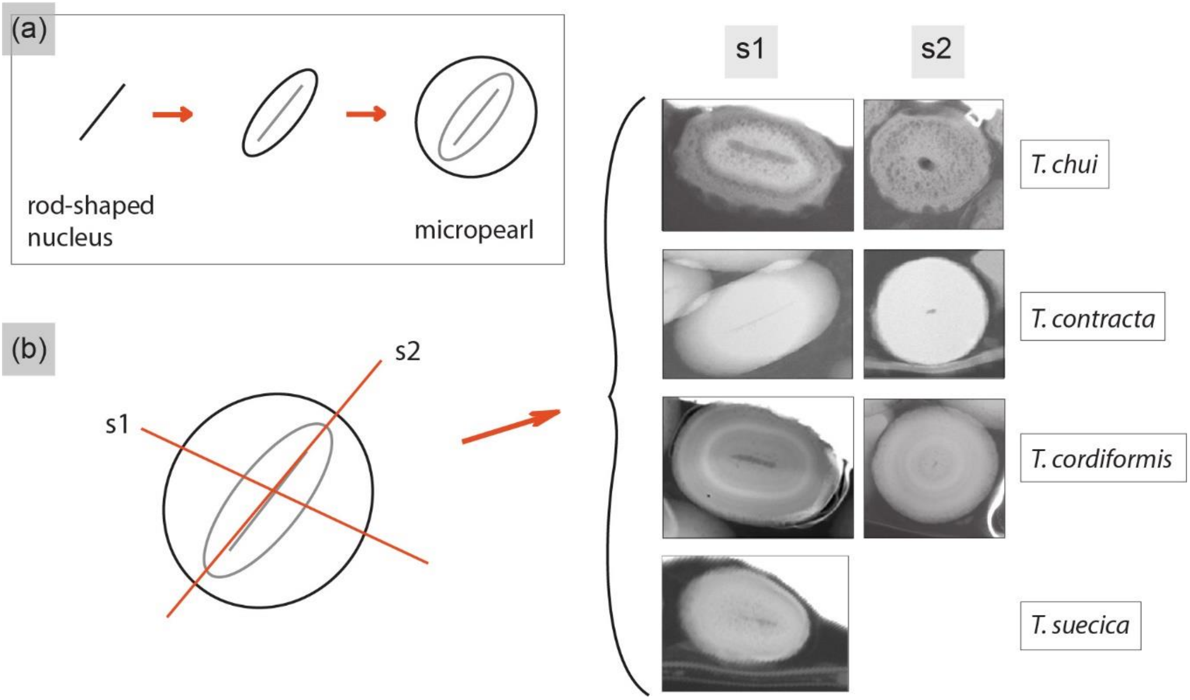
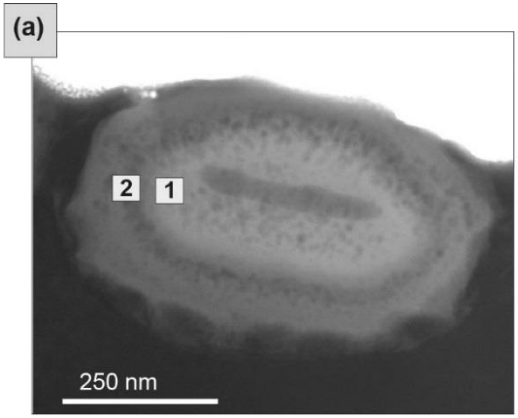


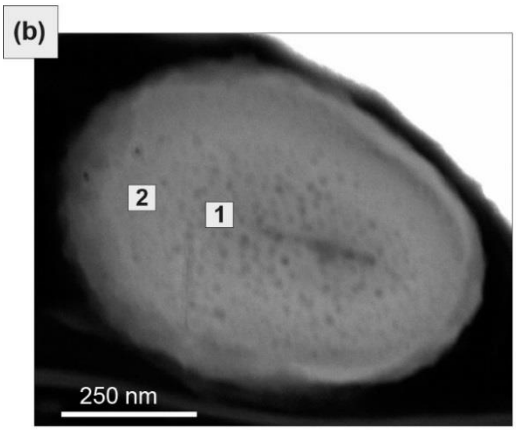
Figure S3: Nucleus shape interpretation for micropearls formed by *Tetraselmis* species.

Cells from algal cultures except *T.*cf *cordiformis,* which was sampled in Lake Geneva (dried samples). (a) Diagram of a possible formation process starting from a rod-shaped nucleus. (b) TEM HAADF images of FIB-cut sections in micropearls formed by *Tetraselmis* species, corresponding to the cross-sections located on the left hand-side drawing. The micropearls measure between 0.7 (*T. chui*) to 1.2 µm (*T. contracta*) in length.



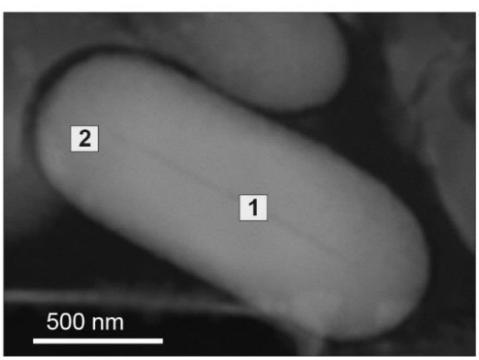
Tetraselmis chui

Element	1: bright	2: dark
C	19.2	19.4
Ca	11.2	17.8
Sr	10.0	3.2
O	59.6	59.7



Tetraselmis suecica

Element	1: internal	2: external
C	21.9	19.9
Ca	8.3	10.2
Sr	8.8	9.9
O	61.0	60.0



Tetraselmis contracta

Element	1	2
C	24	20
K	1	1
Ca	14	20
O	61	59

Figure S4: TEM-EDXS analyses of *T. contracta, T. chui and T. suecica* micropearls composition.

STEM – HAADF image. The location of the EDXS analyses (right hand-side table) is indicated by the corresponding numbers. Results are normalized to 100 at%. O is calculated stoichiometrically based on the cation concentrations. Notice the low but significant presence of K in the micropearl composition of *T. contracta*. However its analysis n°1 does not fulfil carbonate stoichiometry, which may be due to the excess C from organic matter. Note that the calculation mode for the analyses presented in this figure differ from those presented in the rest of the manuscript, as C and O are included in the composition.

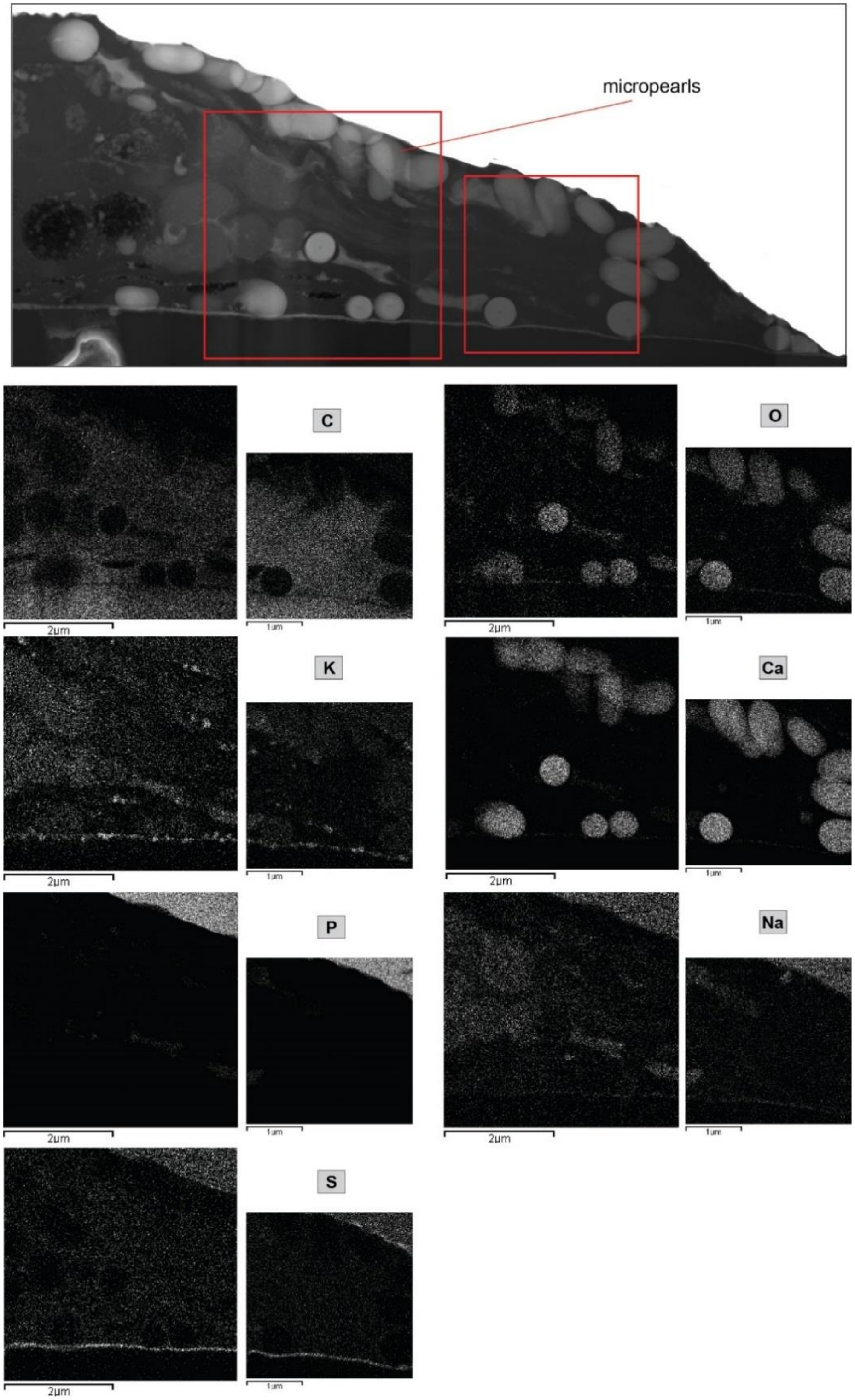


Figure S5: TEM-EDXS mapping results performed on a FIB-cut section through a *Tetraselmis contracta* cell (dried culture sample).

Top image shows the location of the two mappings on a TEM-HAADF image of the section. The maps show the concentration of the different elements: the lighter the color, the more the element is concentrated in that point. Micropearls are mainly composed of Ca, with small quantities of K (and Mg, not shown here). The ACC appears to contain less carbon than the surrounding organic matter, because calcite is known to contain 12 wt% of carbon while the biomass contains 40-50%. Note that, due to the overlap between the P *K* peak and secondary Pt *L* peak, the Pt which was deposited on top of the sample during FIB processing is also visible in green.

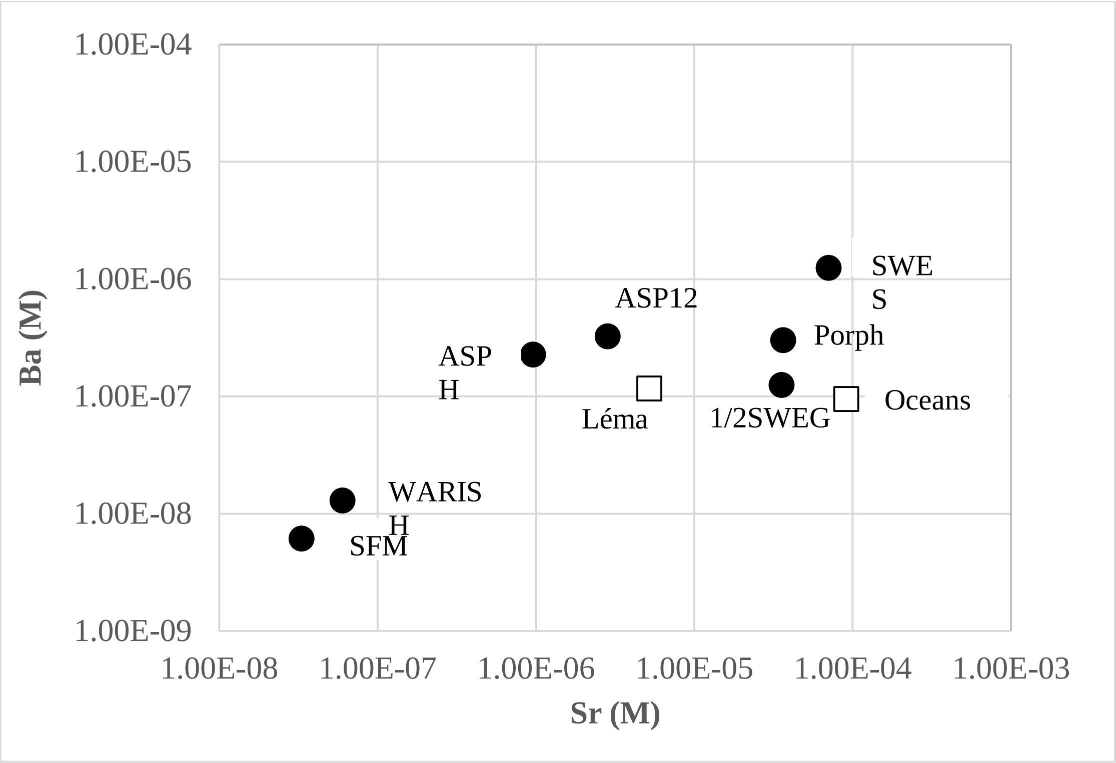


Figure S6: Growth media concentrations in Sr and Ba.

Black dots: culture media; white squares: natural waters. Lake Geneva (Jaquet et al. 2013) and oceans (Bruland and Lohan, 2003). Notice the log scale.

References:

Bruland, K.W. and Lohan, M.C.: Controls of Trace Metals in Seawater, In: Treatise on Geochemistry, Volume 6. Elsevier, chapter 6.02, 2003.

Martignier, A., Pacton, M., Filella, M., Jaquet, J. M., Barja, F., Pollok, K., Langenhorst, F., Lavigne, S., Guagliardo, P., Kilburn, M. R., Thomas, C., Martini, R. and Ariztegui, D.: Intracellular amorphous carbonates uncover a new biomineralization process in eukaryotes, Geobiology, 15, 240–253, doi:10.1111/gbi.12213, 2017.

Provider	Internet address
CCAC Culture Collection of Algae at the University of Cologne	http://www.ccac.uni-koeln.de/sidebar/growth-media/
SAG Sammlung von Algenkulturen at the University of Göttingen	http://www.uni-goettingen.de/en/list+of+media+and+recipes/186449.html
AC Algobank Caen University	https://www.unicaen.fr/algobank/infos/recettes.html

Table S1: Sources for the composition of culture media.

	algal collection	dilution	Sr88(LR) [ppb]	Ba138(L) [ppb]	Sr [M]	Ba [M]	Sr /Ca
Blank - MilliQ_Ge	-		2,48	2,71			
Blank - H2O	CCAC		0,68	0,37			
Blank - MilliQ	SAG		23,43	4,14			
Temoin_10ppb	-		9,89	9,83			
Temoin_100ppb	-		99	103,06			
Waris_H	CCAC		5,27	1,77	6.01E-08	1.29E-08	**1.42E-04**
SFM	CCAC		2,91	0,84	3.32E-08	6.12E-09	**1.58E-04**
1_2_SWEG	SAG		3122,6	17,11	3.56E-05	1.25E-07	**6.77E-03**
ASP-12	CCAC	100	249,47	44,42	2.85E-06	3.23E-07	**2.71E-04**
ASP-H	CCAC	100	84,22	31,09	9.61E-07	2.26E-07	**3.84E-04**
Porph	SAG	100	3196,8	41,38	3.65E-05	3.01E-07	**6.93E-03**
SWES	SAG	100	6201,34	170,84	7.08E-05	1.24E-06	**6.72E-03**

Table S2: Concentration of Sr and Ba measured in the growth media.

Sr and Ba: ICP-MS data.Ca concentrations were calculated based on the media theoretical composition. CCAC: Culture Collection of Algae at the University of Cologne (Germany); SAG: Sammlung von Algenkulturen of the University of Göttingen (Germany); Algobank: culture collection of microalgea of the University of Caen (France). Media ES (Algobank) and Diat (SAG) were not available for analysis.

	cord-F_cc	convol_cc	contract_cc	cord-M_cc	chui_cc	cord_M_sa	cord_Gen	desika_cc	subcord_sa	chui_sa	striata_sa	tetrah	levis_ac	suecica_ac	
Ca norm	0.99	0.99	0.99	0.99	0.99	0.98	0.93	0.90	0.83	0.85	0.77	0.72	0.60	0.49	
Sr norm	0.01	0.01	0.01	0.01	0.01	0.02	0.07	0.08	0.17	0.15	0.23	0.28	0.40	0.51	
Sr/Ca															Total
N	48	22	23	68	48	21	70	22	42	31	38	33	29	33	528
Min	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.042	0.161	0.374	0.490	
Max	0.013	0.050	0.015	0.046	0.025	0.087	1.728	0.154	0.611	0.734	0.667	0.631	1.060	2.620	
Mean	0.005	0.015	0.006	0.008	0.013	0.019	0.118	0.090	0.218	0.199	0.320	0.391	0.701	1.152	
Std. error	0.0005	0.0037	0.0010	0.0008	0.0009	0.0041	0.0369	0.0096	0.0183	0.0306	0.0263	0.0181	0.0394	0.0925	
Stand. dev	0.0038	0.0175	0.0046	0.0067	0.0064	0.0190	0.3090	0.0449	0.1187	0.1705	0.1618	0.1042	0.2120	0.5311	
Median	**0.005**	**0.006**	**0.006**	**0.007**	**0.013**	**0.015**	**0.025**	**0.085**	**0.208**	**0.225**	**0.290**	**0.376**	**0.661**	**1.030**	
25 prcntil	0.001	0.000	0.002	0.004	0.010	0.010	0.004	0.060	0.178	0.040	0.179	0.320	0.545	0.735	
75 prcntil	0.008	0.030	0.010	0.011	0.018	0.024	0.057	0.133	0.260	0.290	0.476	0.469	0.902	1.465	
Coeff. var	73	116	73	81	49	98	262	50	54	85	51	27	30	46	
Medium	SFM	**ASP-H**	**ASP-H**	**Waris-H**	**ASP-H**	Diat	**Lake water**	**ASP-H**	**Porph Ag**	**1/2 SWEg Ag**	**SWES Ag**	**Porp Ag**	ES	ES	
Sr/Ca med	1.58E-04	3.84E-04	3.84E-04	1.42E-04	3.84E-04		4.73E-03	3.84E-04	6.93E-03	6.77E-03	6.72E-03	6.93E-03			
Sr/Ca mp	5.23E-03	5.50E-03	6.28E-03	7.23E-03	1.25E-02	1.48E-02	2.41E-02	8.43E-02	2.08E-01	2.25E-01	2.90E-01	3.76E-01	6.61E-01	1.03E+00	
Enrichment	**33**	**14**	**16**	**51**	**33**		25	**219**	**30**	**33**	**43**	**54**			

Table S3: Composition statistics for micropearls formed by *Tetraselmis* species.

Composition statistics for 13 Tetraselmis strains micropearls. Values from Lake Geneva (labelled cord_Gen; *Martignier et al. 2017*) are given for comparison. Ca norm, Sr norm: values normalized to 1.0. In the lower part of the table, Sr/Ca ratios of the micropearls (mp) are compared to the ratios in their growth medium (med). Enrichment = [(Sr/Ca mp) / (Sr/Ca med)].

References:

Martignier, A., Pacton, M., Filella, M., Jaquet, J. M., Barja, F., Pollok, K., Langenhorst, F., Lavigne, S., Guagliardo, P., Kilburn, M. R., Thomas, C., Martini, R. and Ariztegui, D.: Intracellular amorphous carbonates uncover a new biomineralization process in eukaryotes, Geobiology, 15, 240–253, doi:10.1111/gbi.12213, 2017.