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

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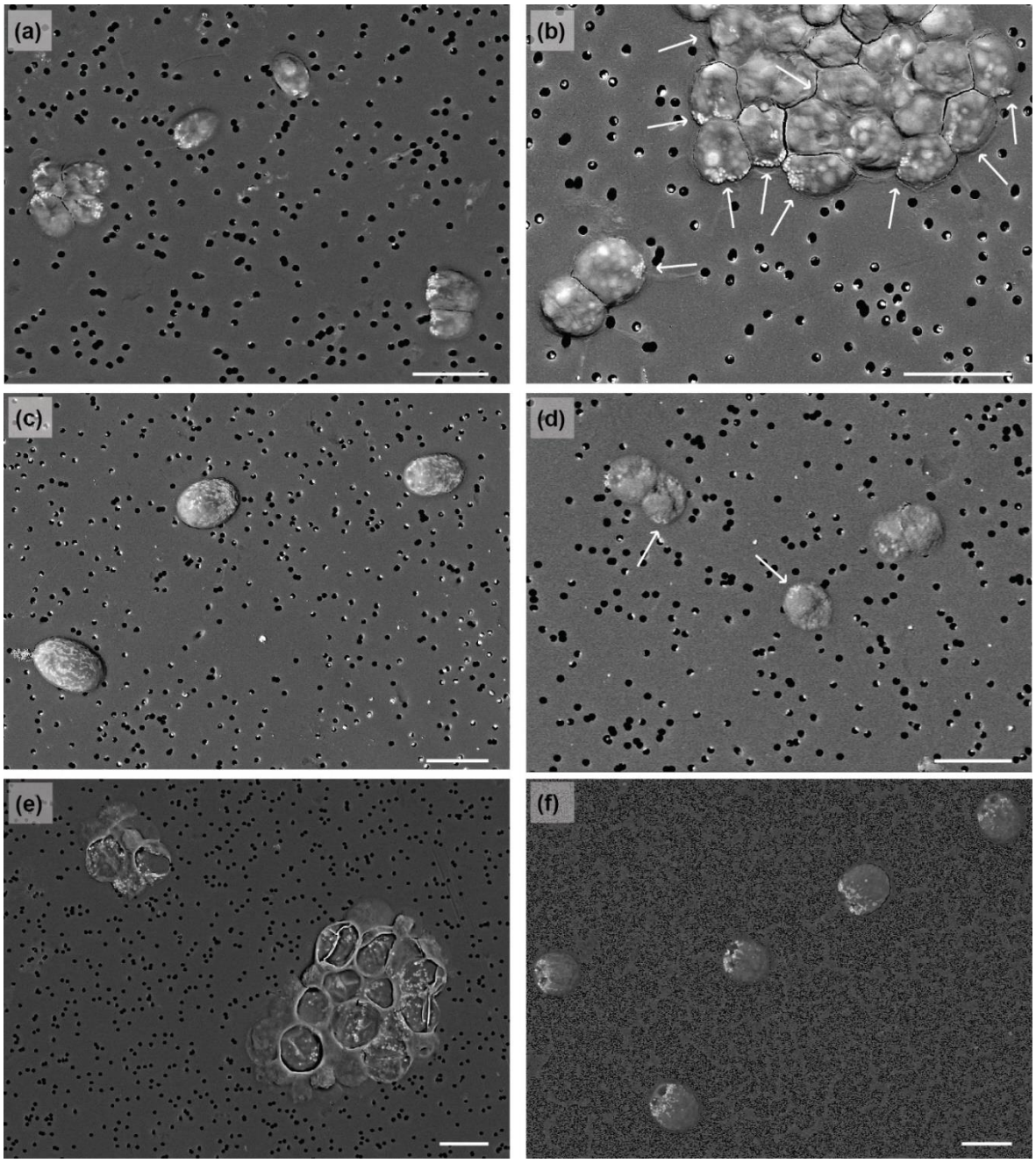

Fig. S1 (part 1): patterns of micropearl arrangement in the different species

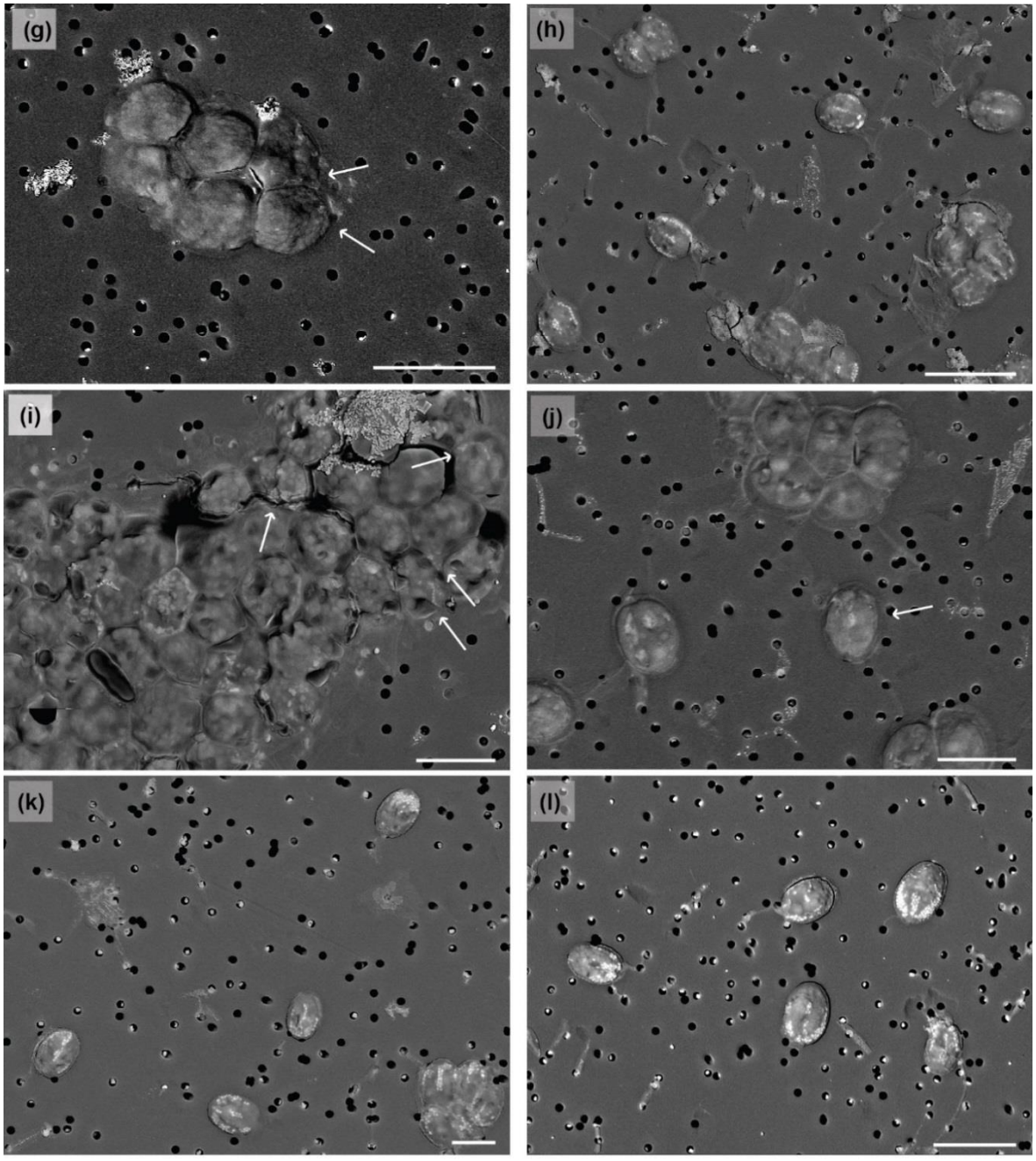

Fig. S1 (part 2): patterns of micropearl arrangement in the different species

SEM backscattered images. (a) *T. chui* (CCAC 0014); (b) *T. chui* (SAG 8-6) ; (c) *T. contracta* (CCAC 1405) ; (d) *T. convolutae* (CCAC 0100) ; (e) *T. cordiformis* (CCAC 0051) ; (f) *T. cordiformis* (CCAC 0579B) ; (g) *T. desikacharyi* (CCAC 0029); (h) *T. levis* (AC 257) ; (i) *T. striata* (SAG 41.85) ; (j) *T. subcordiformis* (SAG 161-1a) ; (k) *T. suecica* (AC 254) ; (l) *T. tetrathele* (AC 261).

These images aim to illustrate the general aspect of the different strains at the time of observation. They correspond to the measurements presented in Table 2. Micropearls appear as white dots inside the cells. In the images where only a few cells contain micropearls, white

arrows indicate their position. Note that polyphosphate inclusions (e.g. in (d)) or NaCl crystals (e.g. in (j)) can also appear as white dots. Their distinction was based on their close-up morphology or EDXS analyses. All images were made on the day following the reception of the strains from the provider, except (f), taken 60 days after reception. This exception allows to show the internal pattern of micropearls in *T. cordiformis*,- pattern that was destroyed during our first sample preparation. Note that strains (b), (i) and (j) were maintained on agar, unlike the other strains. Scale bars: 20 μm.

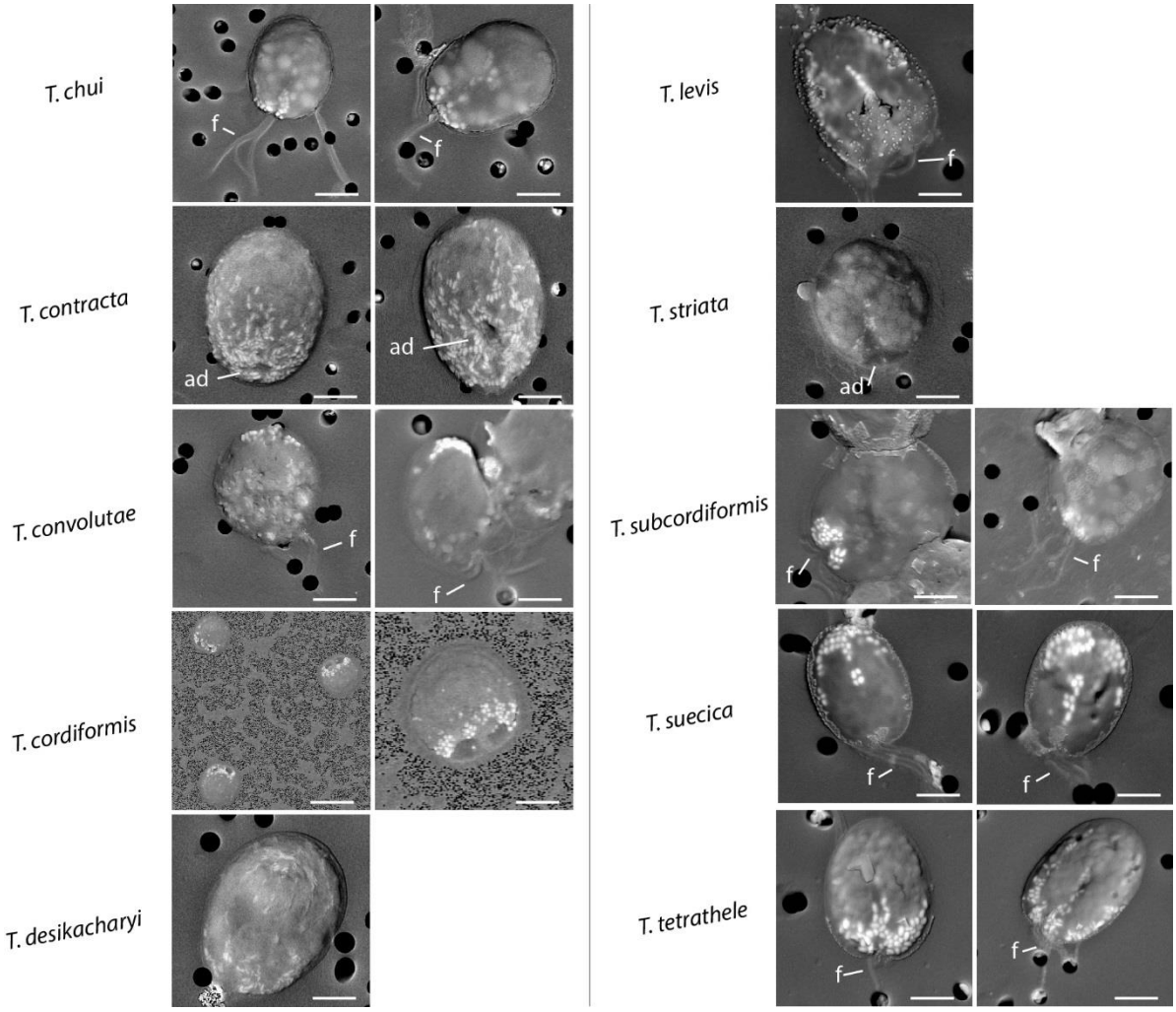

Figure S2: SEM images providing an overview of the micropearl location in *Tetraselmis* species.

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 μm 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 iron oxide minerals (in white) are formed around the (missing) flagella. Scale bars: 5 μm.

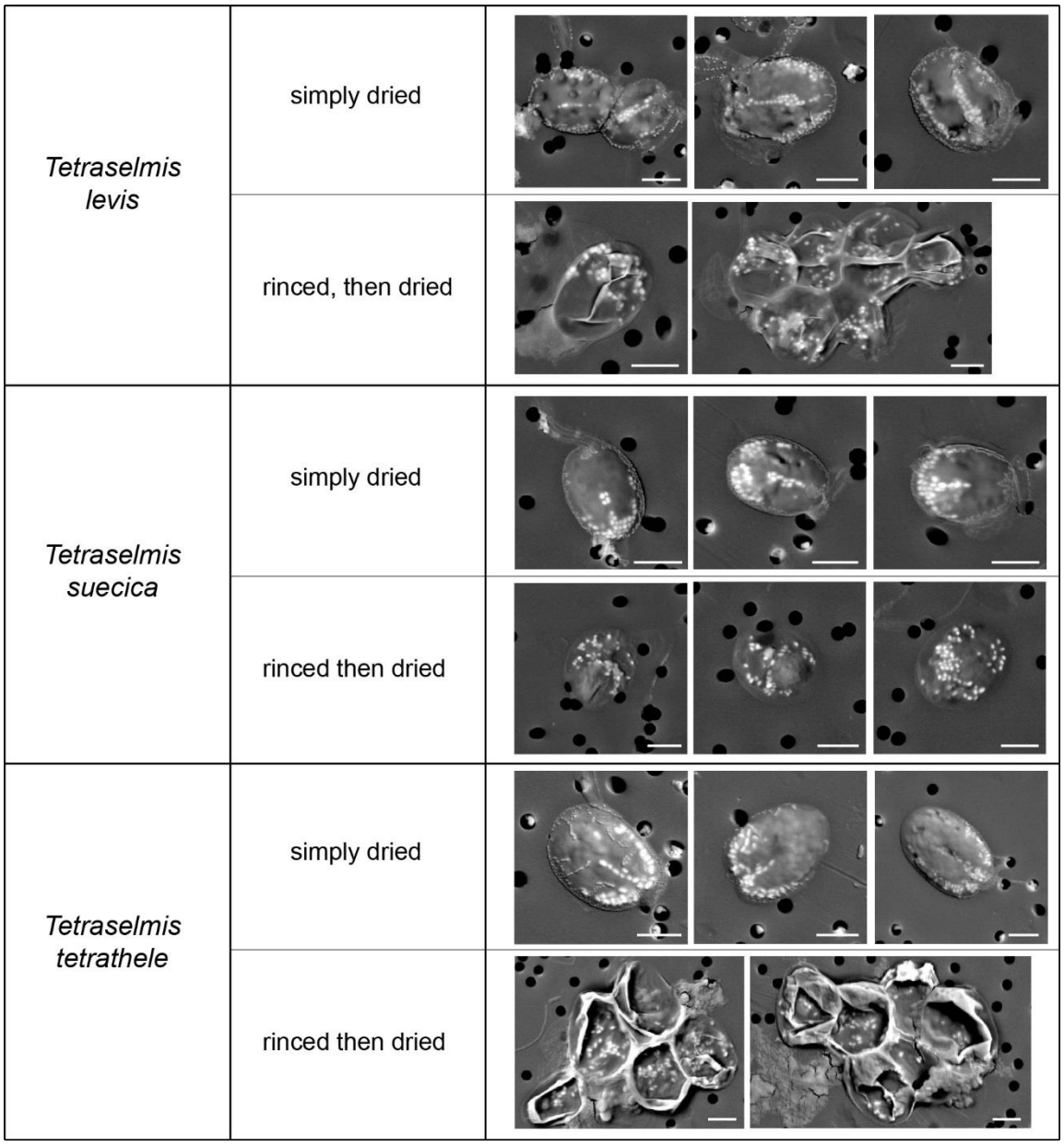

Figure S3: Micropearl distribution inside the cell disrupted by MilliQ water rinsing.

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, or 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.

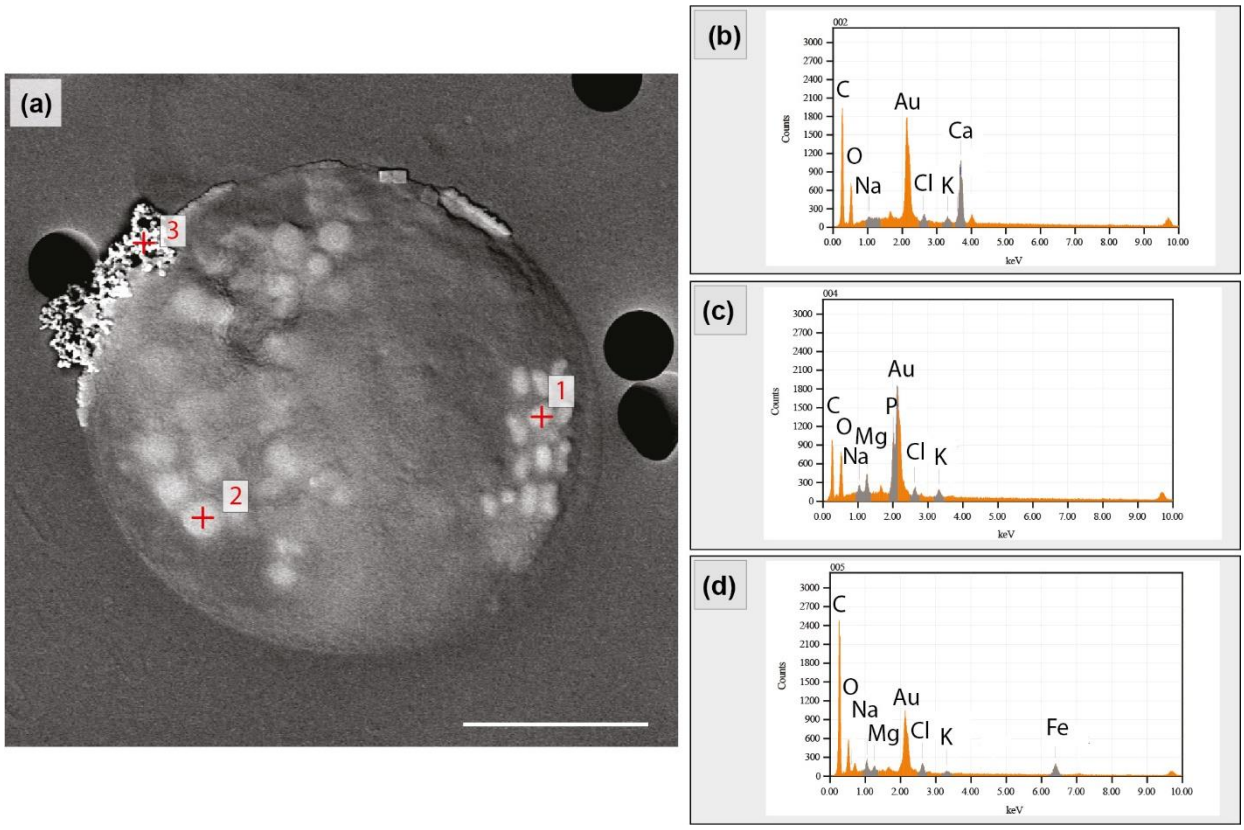

Fig. S4: SEM-EDXS analyses of polyphosphate inclusions and iron oxide aggregate

(a) SEM backscattered image of T.convolutae (Fig. 1c) with localization of the EDXS analyses indicated in red. (b) SEM-EDXS analyse of micropearls. (c) SEM-EDXS analyse of an intracellular polyphosphate inclusion. (c) SEM-EDXS analysis of an extracellular iron oxide aggregate. Due to the small size of the analysed features, the results may include low percentages of elements coming from the surrounding organic matter (e.g. Na, Cl and possibly K). Presence of gold is due to the coating for SEM observation. Scale bars: 5 µm.

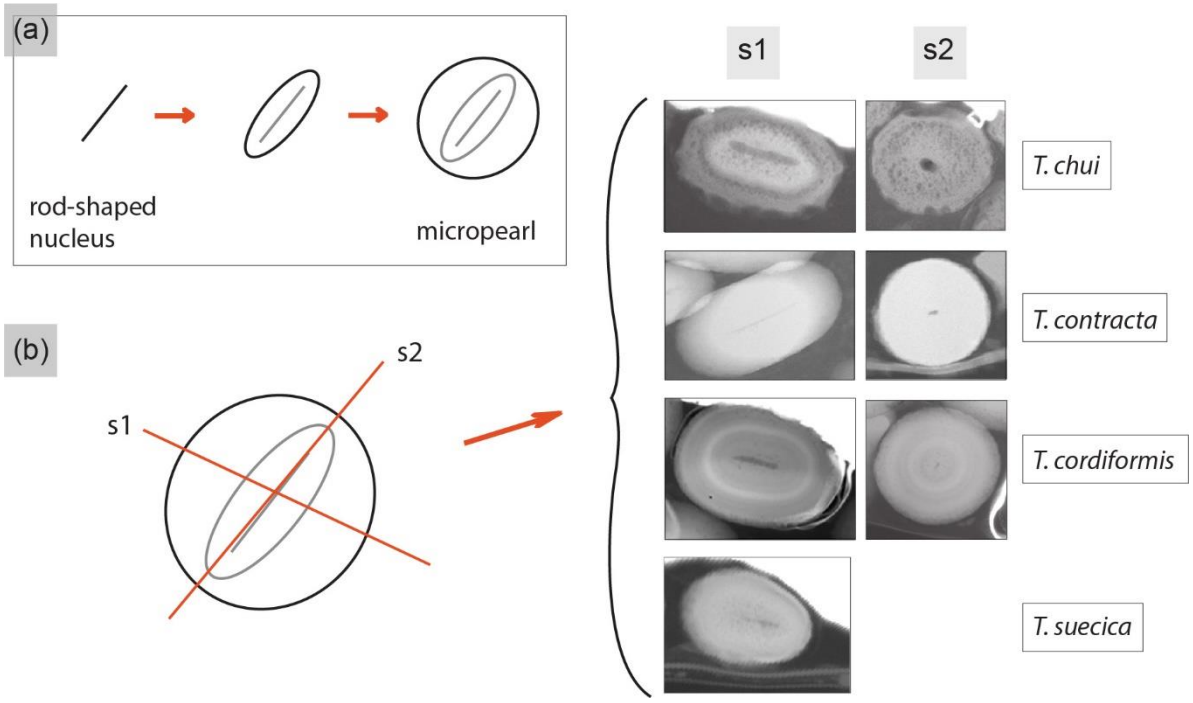

Figure S5: Nucleus shape interpretation for micropearls formed by *Tetraselmis* species.

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.

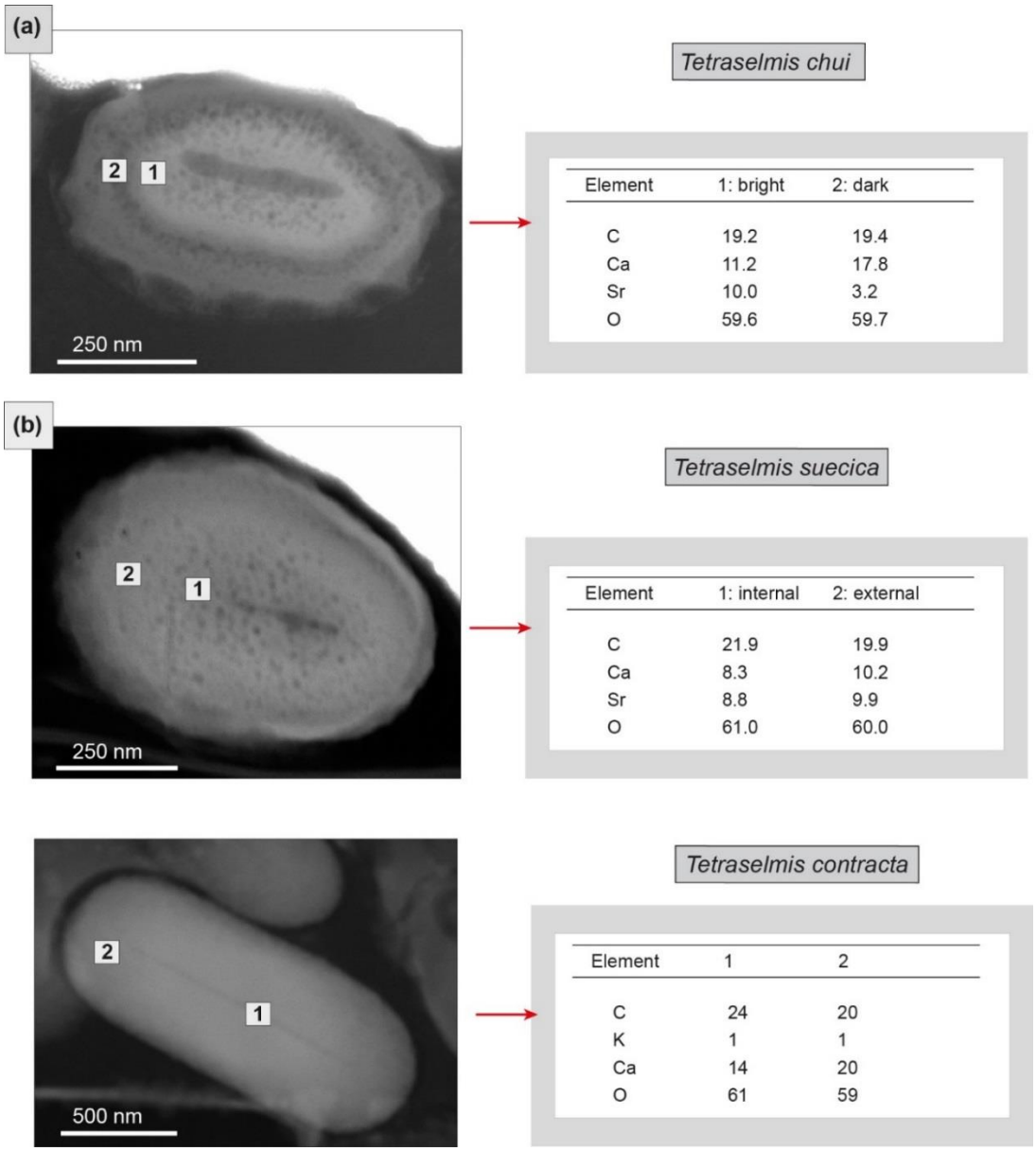

Figure S6: TEM-EDXS analyses of *T. contracta, T. chui and T. suecica* micropearls.

Cut-out of single micropearls (left) from STEM – HAADF images of the FIB section shown in Figs 2 and 3. The location of the EDXS analyses (shown in the 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 (with absorption correction for sample thickness). (a) The micropearl of *T. chui* shows a clear zonation which is well documented in the TEM-EDXS results. (b) The micropearl of *T. suesica* appears almost unzoned, but with a high Sr concentration (Sr/Ca close to 1). Two additional thin zones close to the rim are visible but they are too small to allow meaningful TEM-EDXS analyses.(c) *T. contracta* has a very low concentration of Sr (close to or under the detection limit) in TEM-EDXS. No zoning was detected. In contrast, a low but significant presence of K was detected. 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 in order to perform a meaningful absorption correction.

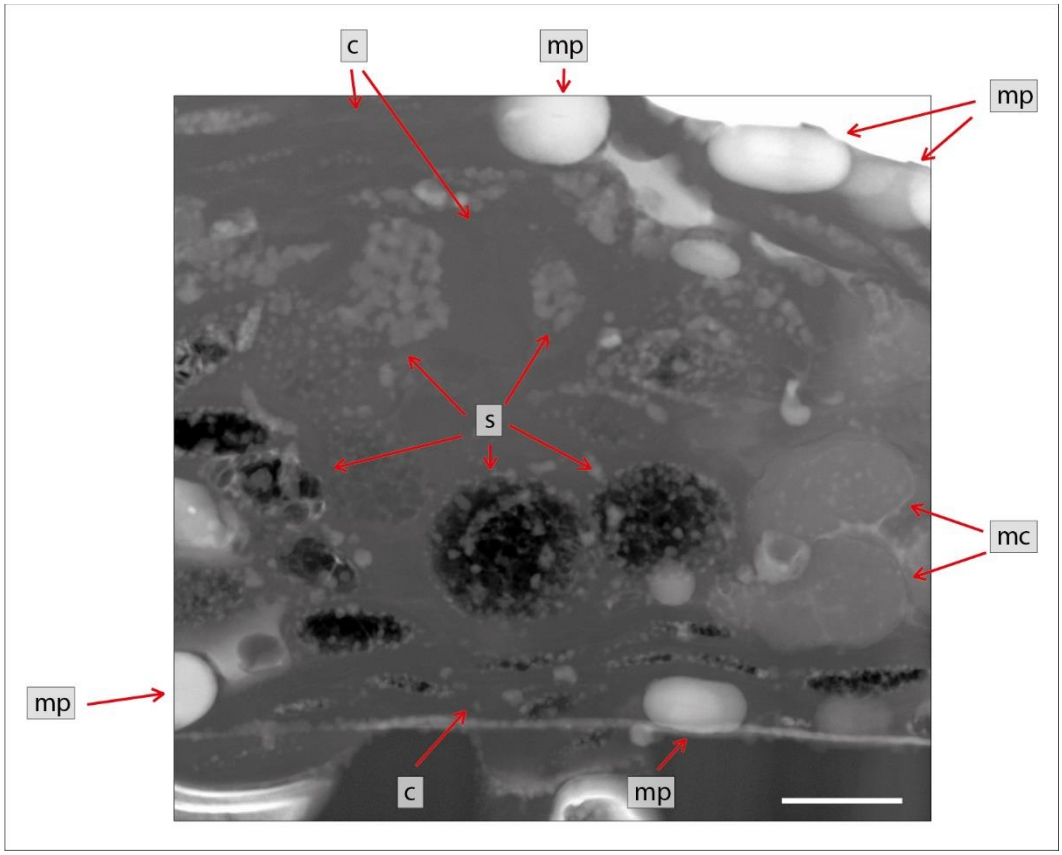

Figure S7: TEM zoom on a FIB-cut section through a *Tetraselmis contracta* cell (dried culture sample).

TEM-HAADF image. Zoomed-in image on a part of Figure 3. The micropearls show light or medium grey shades, regular round or oval shapes. Tentative identification of the visible cellular constituents. s: starch grains; c: chloroplast; mp: micropearls; mc: mitochondria.

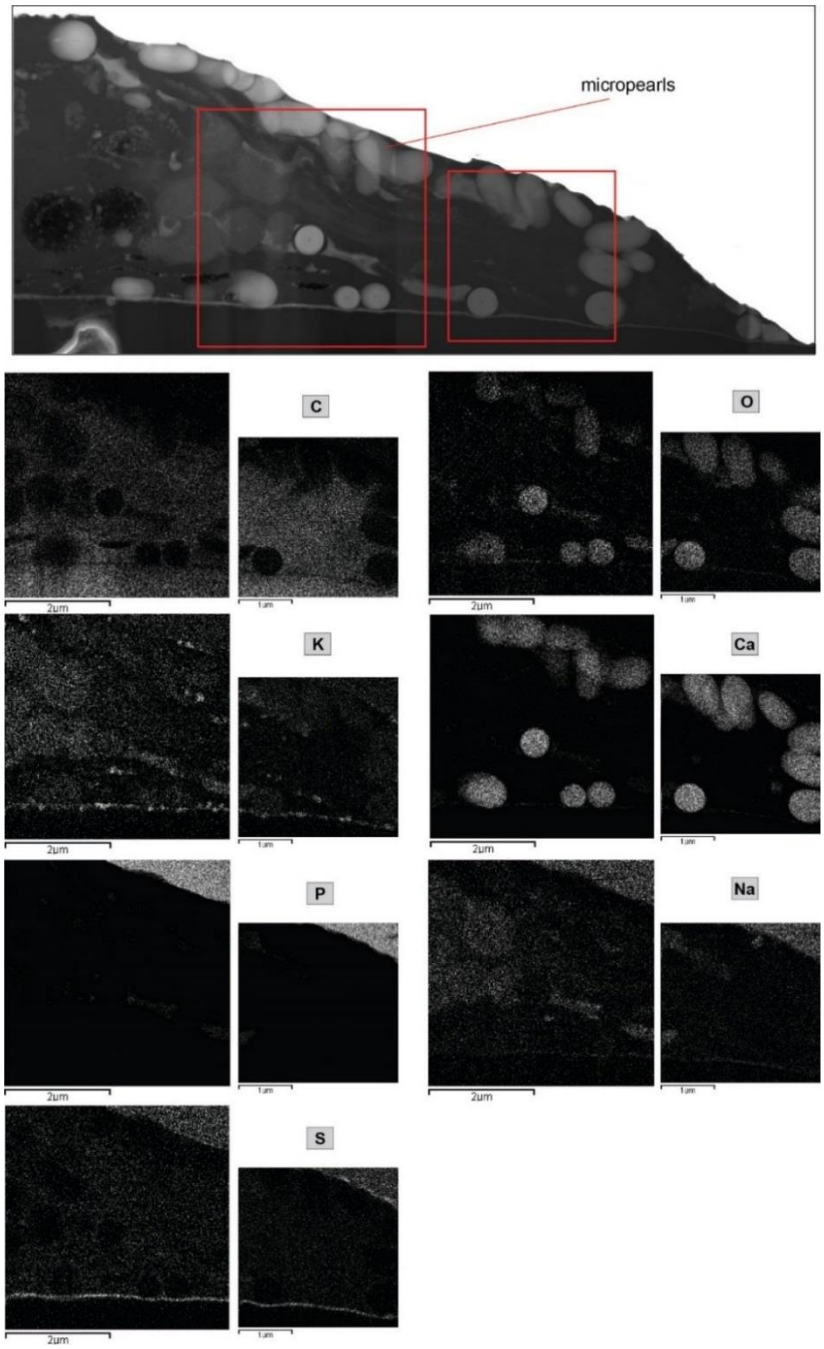

Figure S8: TEM-EDXS mapping results performed on a FIB-cut section through a *Tetraselmis contracta* cell (dried culture sample).

The 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 C than the surrounding organic matter, because calcite is known to contain 12 wt% of C 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.

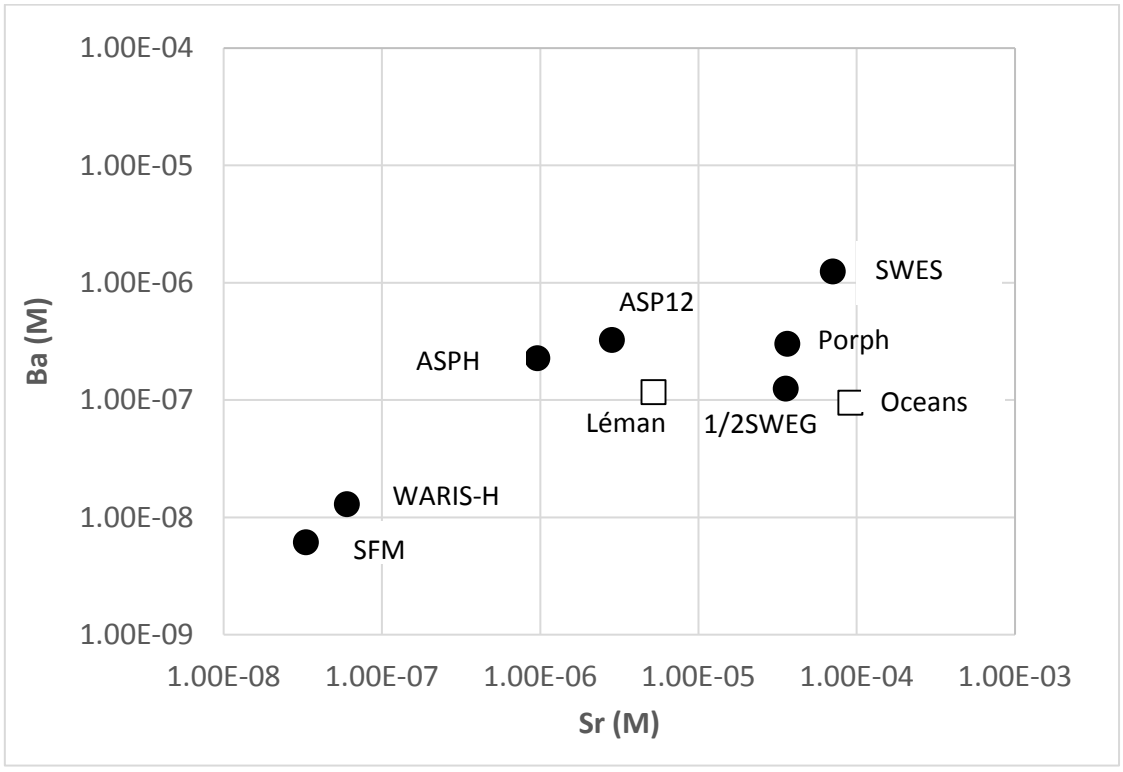

Figure S9: Growth media Sr and Ba concentrations.

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.

Jaquet, J. M., Nirel, P. and Martignier, A.: Preliminary investigations on picoplankton-related precipitation of alkaline-earth metal carbonates in meso-oligotrophic lake Geneva (Switzerland), J. Limnol., 72, 592–605, doi:10.4081/jlimnol.2013.e50, 2013.

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 of 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.

Micropearls	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	tetrath_ac	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														
N	48	22	23	68	48	21	70	22	42	31	38	33	29	33
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**	**ES**	**ES**	**ES**
Sr/Ca medium	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	9.00E-03	9.00E-03	9.00E-03
Sr/Ca mpearls	5.23E-03	5.50E-03	6.28E-03	7.23E-03	1.25E-02	1.48E-02	5.82E-02	8.43E-02	2.08E-01	2.25E-01	2.90E-01	3.76E-01	6.61E-01	1.03E+00

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)]. The concentrations for the ES medium (not analyzed) were set as equivalent to standard sea water, ie. Sr=9 10-5 M. Ca=10-2 M, giving a Ratio Sr/Ca= 9 10-3.

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.

E factor	Strain	Medium	*E* factor	Environment
	cord_L	Lake Geneva	12	Freshwater
Low	*convol_cc*	ASP-H	14	Marine symbiotic
	contract_cc	ASP-H	16	Brackish
	subcord_sa	Porph Ag	30	Marine
Mdium	*cord-F_cc*	SFM	33	Freshwater
	chui_cc	ASP-H	33	Marine
	chui_sa	1/2 SWEg Ag	33	Marine
	tetrath	ES	42	Brackish
High	*striata_sa*	SWES Ag	43	Marine
	cord-M_cc	Waris-H	51	Freshwater
Very High	*desika_cc*	ASP-H	219	Marine sand

Table S4: Ranking of the Enrichment (E) factor amongst species.

Enrichment factor (E) was calculated as the molar ratio [(Sr micropearls / Ca micropearls) / (Sr medium / Ca medium)], on the basis of the data shown in Table S2 and Table S3.