Supplementary Material: Specific Chemical and Genetic Markers Revealed a Thousands-Year Presence of Toxic *Nodularia spumigena* in the Baltic Sea

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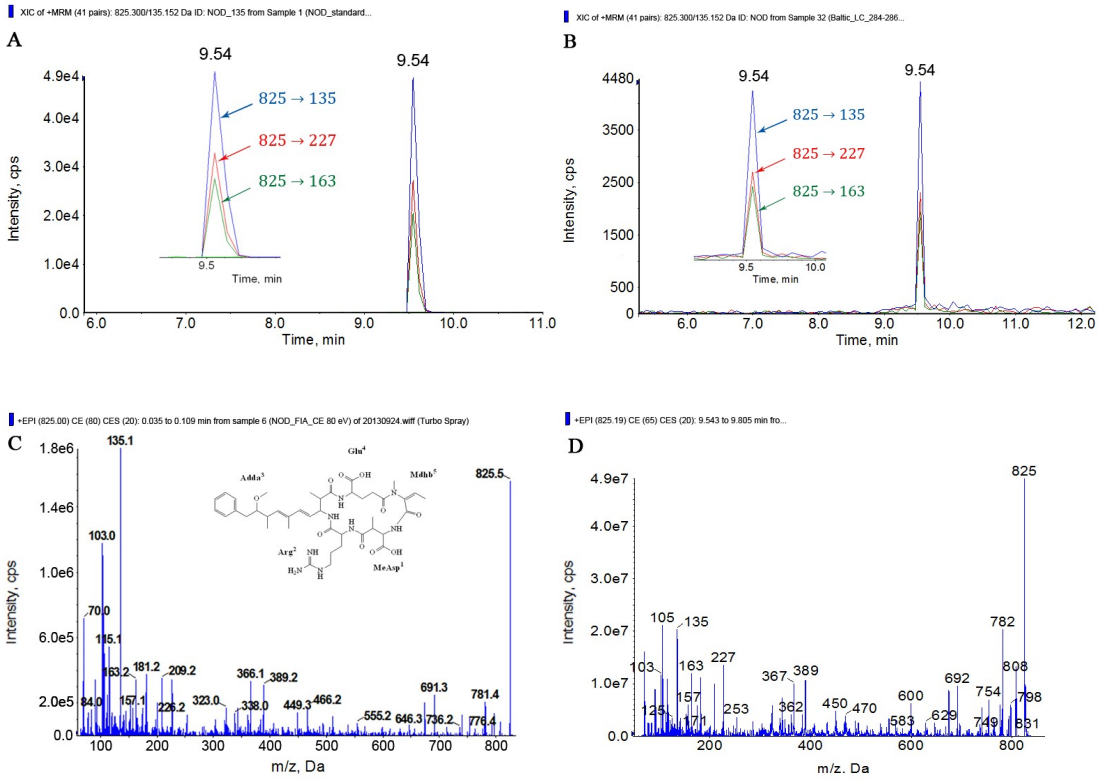

Figure S1: MRM chromatograms of nodularin (NOD) standard (A) and NOD extracted from 284–286 cm layer of long sediment core (LC) collected in the Gulf of Gdańsk, Southern Baltic Sea (B); the MRM transitions are marked in different colors. Chemical structure and enhanced ion product mass spectra (EPI) of nodularin standard (C) and EPI of NOD extracted from 284–286 cm layer of LC (D).

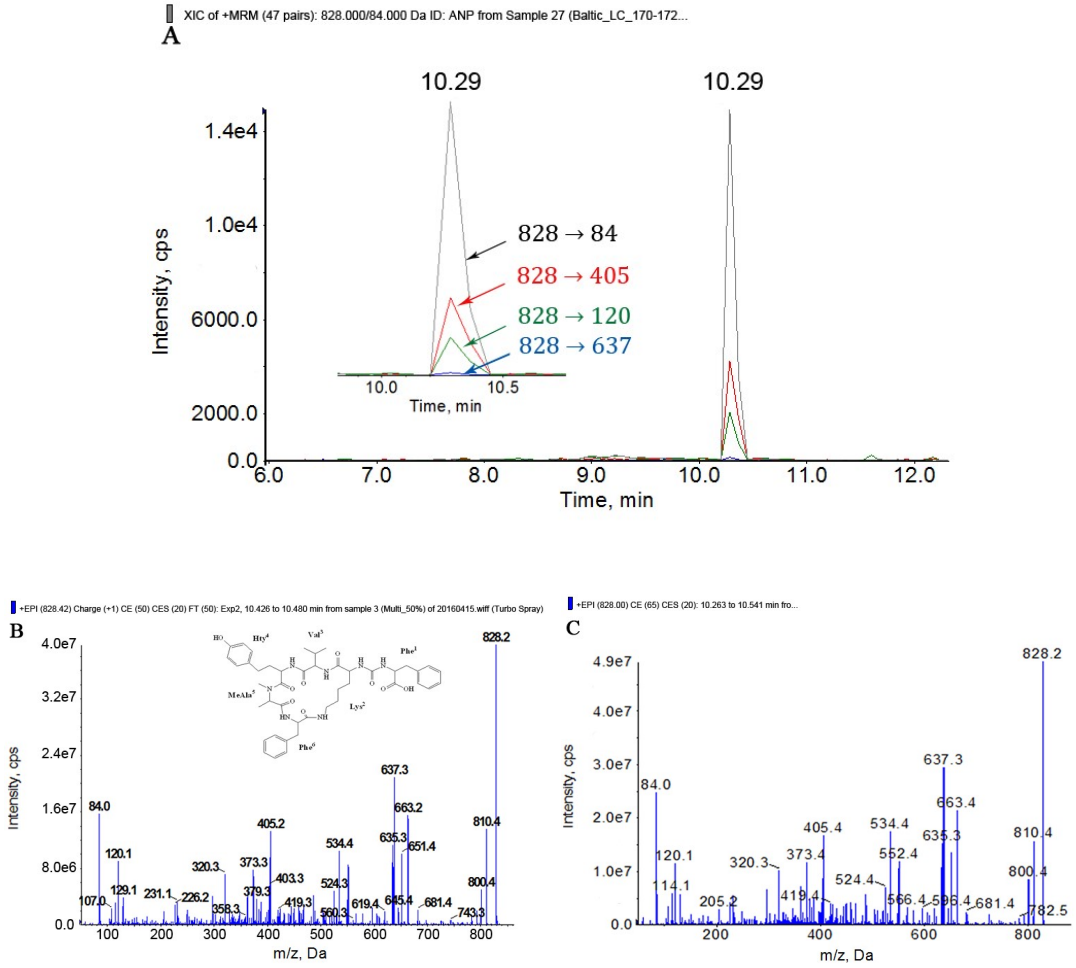

Figure S2: MRM chromatogram of anabaenopeptin AP827 extracted from 170–170 cm layer of long sediment core (LC) collected in the Gulf of Gdańsk, Southern Baltic Sea; the MRM transitions are marked in different colors (A). Chemical structure and enhanced ion product mass spectra of anabaenopeptin AP827 extracted from *N. spumigena* CCNP1401 (B) and EPI of AP827 170–170 cm layer of LC (C).

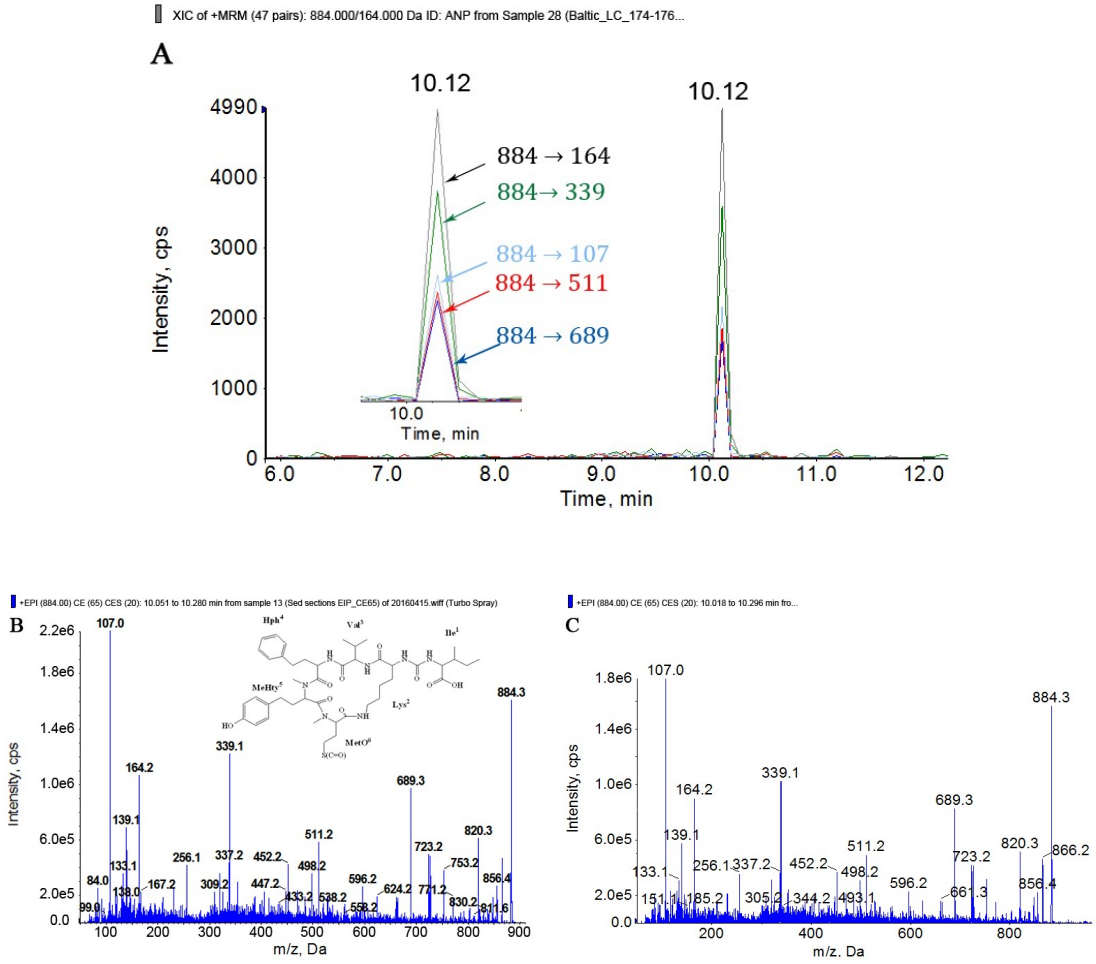

Figure S3: MRM chromatogram of anabaenopeptin AP883a extracted from 174–176 cm layer of long sediment core (LC) collected in the Gulf of Gdańsk, Southern Baltic Sea; the MRM transitions are marked in different colors (A). Chemical structure and enhanced ion product mass spectra of anabaenopeptin AP883a extracted from *N. spumigena* CCNP1402 (B), and EPI of AP883a extracted from 174–176 cm layer of LC (C).

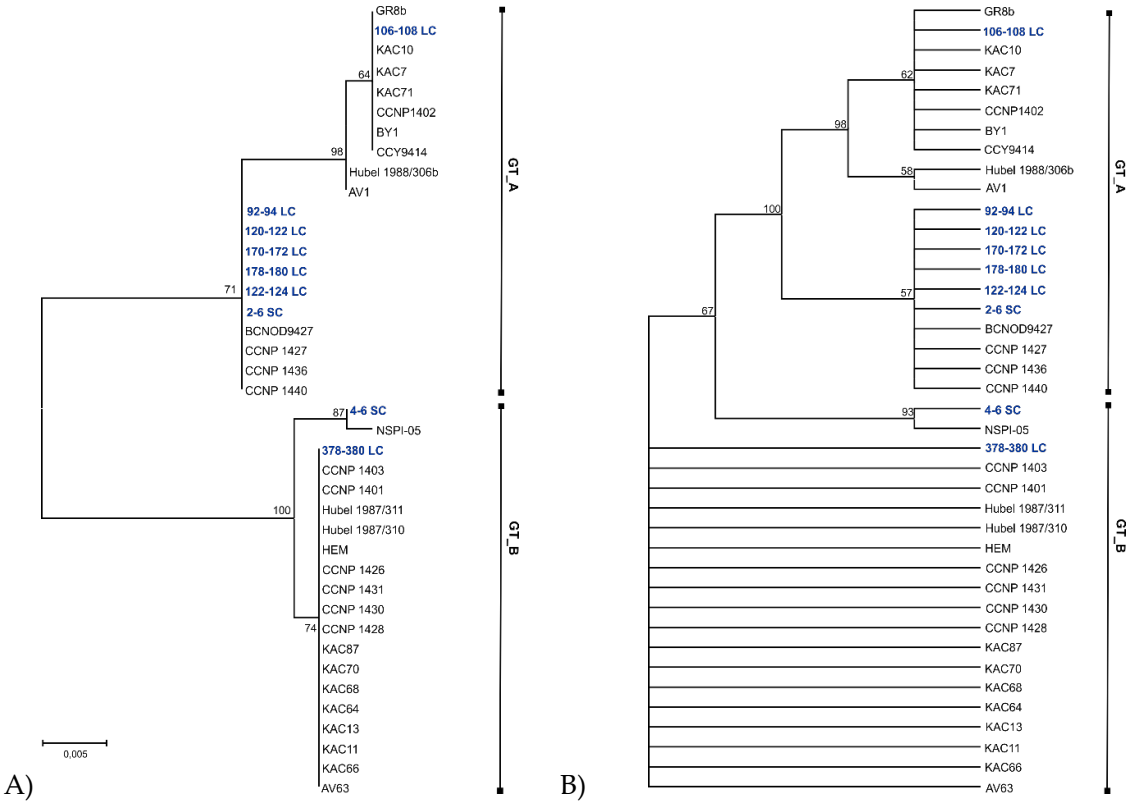

Figure S4: Neighbour-joining (NJ) (A) and Maximum parsimony (MP) (B) phylogenetic trees based on the *cpcBA*-IGS sequences (496 bp) obtained from DNA isolated from Baltic sediments (marked in blue) and reference *cpcBA*-IGS sequences (retrieved from NCBI) from *N. spumigena* strains (marked in black). Phylogenetic relationships were bootstrapped 1000 times. The branches with less than 50% bootstrap are shown as unresolved. Similar *cpcBA*-IGS sequences are marked as GT A and GT B, respectively (**GT**, genotype).

Table S1: Changes in nodularin concentrations [ng/g dw] and changes in relative amounts of anabaenopeptins (expressed as a ratio of AP peak area/g dw) in short (SC) and long core (LC).

Core layer	NOD concentration [ng/g]	AP827 [peak area/g]	AP883a [peak area/g]
Short core (SC)			
0–2	1.05	0	0.36
2–4	1.63	0.72	0.44
4–6	4.32	0.76	0.36
6–8	1.45	0.25	0.54
12–14	2.47	0	0.83
14–16	0.12	0.2	0
16–18	0.18	0	0
18–20	0.13	0.5	0
20–22	0.11	0.4	0
22–24	0.14	0.16	0
24–26	0.27	0.27	0
26–28	0.38	0.27	0
28–30	0.12	0.28	0
Long core (LC)			
0–2	0.23	0.13	0
2–4	0.15	0.11	0
4–6	0.23	0.28	0
6–8	0.21	0.24	0
8–10	0.24	0.39	0.12
10–12	0.26	0.27	0
12–14	0.23	0.27	0
14–16	0.30	0.39	0.23
16–18	0.33	0.36	0.21
18–20	0.33	0.26	0.29
20–22	0.34	0.47	0.29
24–26	0.33	0.36	0.13
28–30	0.32	0.41	0.11
32–34	0.63	0.82	0.6
36–38	0.67	0.72	0.42
40–42	0.73	0.93	0.56
44–46	0.68	1.04	0.52
48–50	0.68	0.67	0.3
52–54	0.74	0.71	0.44
56–58	0.62	0.76	0.29
60–62	0.61	0.74	0.42
64–66	0.61	0.73	0.29
68–70	0.55	0.58	0.46
72–74	0.61	0.64	0.39
76–78	0.58	0.72	0.4
78–80	0.74	1.25	0.19
80–82	2.52	1.66	1.61
82–84	4.84	16.2	1.42
84–86	1.68	2.82	1.27
86–88	8.96	6.01	1
88–90	2.83	3.85	1.94
90–92	29.98	31.4	6.68
92–94	1.87	2.33	1.38
94–96	1.86	0.93	0
96–98	2.08	1.72	1.5
98–100	13.48	13.95	1.05
100–102	1.92	1.73	1.43
102–104	1.29	1.56	0.38
104–106	1.56	1.24	0.91
106–108	31.44	24.62	6.66
108–110	1.27	0.98	0.79
110–112	1.09	0.98	1.01
112–114	1.35	2.14	0.93
114–116	10.13	6.91	3.91
116–118	1.42	1.45	1
118–120	7.10	10.22	2.03
120–122	7.83	1.43	1.07
122–124	31.31	23.49	4.86
124–126	1.24	1.12	0.86
126–128	0.55	1.26	0
128–130	0.64	0.81	1.93
130–132	1.94	12.29	4.08
132–134	1.71	1.34	2.52
134–136	1.13	0.53	4.3
136–138	1.29	1.53	2.97
138–140	3.00	3.06	1.26
140–142	0.99	1.06	1.07
142–144	1.04	1.11	0
144–146	0.70	0.95	0.35
146–148	0.35	0.11	0
148–150	1.24	1.67	1.06
150–152	0.48	0.14	0
152–154	0.27	0.74	0.2
154–156	0.51	0.5	0
156–158	0.34	0.5	0
158–160	0.96	1.97	0.87

Core layer	NOD concentration [ng/g]	AP827 [peak area/g]	AP883a
Long core (LC)			
160–162	0.46	1.02	0.12
162–164	0.21	0	0
164–166	0.46	0.61	0.33
166–168	0.91	1.09	0
168–170	0.31	0.97	0.15
170–172	225.04	67.77	13.83
172–174	0.52	0.6	0.53
174–176	185.00	57.07	20.6
176–178	0.41	1.17	1.06
178–180	49.71	56.4	4.82
180–182	5.95	0.83	1.12
182–184	3.42	2.78	0
184–186	0.30	0.98	0.73
186–188	0.39	0	0
188–190	0.16	0	0
192–194	0.15	2.8	0
196–198	0.19	0.19	0
202–204	0.24	0.16	0
206–208	0.14	0.14	0
210–212	0.27	0.27	0.1
214–216	0.25	0.14	0.14
218–220	0.33	0.26	0
222–224	0.24	0	0
226–228	0.16	0.22	0
230–232	0.15	0.15	0
234–236	0.13	0.22	0
238–240	0.11	0.17	0.11
242–244	0.16	0.18	0
246–248	0.18	0.19	0
250–252	0.24	0.37	0
254–256	0.27	0.33	0.14
256–258	0.28	0.23	0.14
260–262	0.31	0.28	0
264–266	0.46	0.48	0.23
268–270	0.41	0.52	0.12
272–274	0.31	0.28	0.24
276–278	0.35	0.45	0.2
280–282	0.26	0.28	0.21
284–286	0.26	0.73	0.43
288–290	0.09	0.13	0
292–294	0.15	0.17	0
296–298	0.16	0.28	0.19
300–302	0.13	0.57	0
304–306	0.12	0.12	0
308–310	0.10	0.21	0
312–314	0.13	0.27	0
316–318	0.12	0.22	0
320–322	0.12	0.14	0
324–326	0.11	0.35	0
328–330	0.13	0.18	0
332–334	0.14	0.2	0
336–338	0.16	0.16	0
340–342	0.12	0.23	0
344–346	0.08	0.21	0.11
348–350	0.07	0.2	0
352–354	0.10	0.25	0
354–356	0.11	0	0
356–358	0.13	0.32	0
358–360	0.13	0.54	0.21
360–362	0.10	0.32	0
364–366	0.11	0.26	0
368–370	0.12	0.33	0
372–374	0.16	0.32	0
376–378	0.12	0.33	0
380–382	0.14	0.4	0

Table S2: List of sediment samples from short core (SC) and long core (LC) and type of genetic analysis done in the work (+ indicates type of the analysis done with use of selected sediment sample, ++ indicates presence of selected PCR product).

Sediment layer [cm]	PCR (*nda*F/*mcy*E)		PCR (PC-IGS)		PC-IGS sequences accession numbers
	analyzed	detected	analyzed	detected	
Short core (SC)					
0–2	+	++	+	++	
2–6			+	++	MF101236
4–6	+	++	+	++	MF101243
Long core (LC)					
2–4	+	++	+	++	
4–6	+	++	+	++	
6–8	+	++	+	++	
10–12	+	++	+	++	
12–14	+	++	+	++	
14–16	+	++	+	++	
84–86			+	++	
92–94			+	++	MF101240
106–108			+	++	MF101241
114–116			+	++	
120–122			+	++	MF101242
122–124			+	++	MF101237
124–126			+	++	
136–138	+		+	++	
138–140	+		+	++	
166–168	+		+	++	
170–172			+	++	MF101239
178–180	+	++	+	++	MF101238
340–342	+		+	++	
378–380	+		+	++	MF101243

Table S3: The quantity (ng/μL) and quality ($A_{260/280}$) of DNA extracted from selected sediment layers. (**MP** - DNA isolated with FastDNA™ Kit for Soil, **MPA** - DNA isolated with FastDNA™ Kit for Soil and cleaned-up with Anty-Inhibitor Kit, **N1** - DNA isolated with NucleoSpin® Soil using SL1 buffer, **N1A** - DNA isolated with NucleoSpin® Soil using SL1 buffer and cleaned-up with Anty-Inhibitor Kit, **N2** - DNA isolated with NucleoSpin® Soil using SL2 buffer, **N2A** - DNA isolated with NucleoSpin® Soil using SL2 buffer and cleaned-up with Anty-Inhibitor Kit).

DNA amounts [ng/μL]														
	Sediment layer [cm]													
Method	**0–2**	**4–6**	**2–4**	**4–6**	**6–8**	**10–12**	**12–14**	**14–16**	**136–138**	**138–140**	**166–168**	**178–180**	**340–342**	**378–380**
	Short core (SC)							**Long core (LC)**						
MP	266	202	92	80	88	110	68	70	64	93	56	37	24	35
MPA	67	50	24	18	27	37	43	33	23	36	27	15	5	7
N1	184	119	62	84	48	49	16	43	59	34	30	13		16
N1A	100	84	30	38	36	315	29	32	17	15	5			
N2	225	137	6	23	12	19	17	22	38	25	22	8		
N2A	125	104		4	4	11	3	7	11	10	7			

$A_{260/280}$														
	Sediment layer [cm]													
Method	**0–2**	**4–6**	**2–4**	**4–6**	**6–8**	**10–12**	**12–14**	**14–16**	**136–138**	**138–140**	**166–168**	**178–180**	**340–342**	**378–380**
	Short core (SC)							**Long core (LC)**						
MP	1.6	1.5	1.4	1.5	1.5	1.5	1.6	1.6	1.6	1.6	1.6	1.8	2.2	2.0
MPA	1.4	1.3	1.5	1.7	1.8	1.5	1.8	1.7	1.4	1.6	1.6	1.8	2.0	1.8
N1	1.9	1.9	1.8	1.6	1.9	1.8	1.7	1.7	1.4	1.6	1.5	1.3		1.8
N1A	1.8	1.6	1.7	1.7	1.7	1.6	1.9	1.8	1.4	1.6	1.6	1.3		
N2	1.7	1.6	1.5	1.2	1.2	1.1	1.4	1.1	1.6	1.6	1.2	1.3		
N2A	1.8	1.8		1.2	1.2	1.2	1.5	1.1	1.6	1.6	1.2			

Table S4: Exemplary studies on the presence and diversity of cyanobacterial communities in sediment samples conducted with the application of genetic methods. * The oldest analyzed cyanobacterial DNA.

Type of material	Core length [m]	Estimated age of the deepest part of the core [years]	Type of nucleotitede sequences	Seuqnces deposited in GenBank	Genetic method	Reference
Pacific Ocean	surface		16S rRNA	Yes	PCR, DGGE	[46]
Saline lakes, coastal marine basin (Antarctica)	surface		16S rRNA	Yes	Cloning, RFLP	[47]
Freshwater lake (Keyna)	0.10	not known	16S rRNA, *mcyE*/*ndaF*	Yes	PCR-DGGE	[48]
Saline lakes (USA)	0.10	40 BP	*mcyA*, *mcyD*, 16S rRNA	Yes	PCR, qPCR	[49]
Freshwater lakes (France)	0.25	90 BP	16S rRNA-ITS, *mcyA*, PC-IGS	Yes	PCR, qPCR	[50]
Freshwater lake (France)	0.40	100 BP	16S rRNA, ITS 1	Yes	PCR, qPCR	[51]
Freshwater Lagoon (Urugway)	0.50	170 BP	16S-23S - ITS, *sxtU*	Yes	Cloning, PCR, qPCR, DGGE	[52]
Freshwater lakes (Switzerland)	1.00	200 BP	16S rRNA, *mcyA*	Yes	PCR	[53]
Freshwater lakes (Norway)	0.45	400 BP	*ociB*	No	PCR, qPCR	[54]
Freshwater lake (Canada)	0.40	440 BP	16S rRNA, *glnA*	No	PCR, qPCR	[55]
Saline lakes (Antarctica)	2.45	3000 BP	16S rRNA	Yes	PCR-DGGE	[56]
Saline lake (China, Asia)	5.00	3000 BP	23S rRNA	No	PCR, qPCR, DGGE	[57]
Baltic Sea (Europe)	4.00	>4000 BP	16S rRNA, *nadF*/*mcyE*, PC-IGS	Yes	PCR	This work
Baltic Sea (Europe)	5.50	8500-7400 BP	16S rRNA	Yes	Cloning, T-RFLP	[28]
Saline lake (Antractica)	1.50	10 000 BP	16S rRNA	Yes	PCR, qPCR, DGGE	[58]
Saline lake (China)	5.60	18 500 BP	23S rRNA	Yes	PCR-DGGE	[59]
*Marine evaporites (Italy)		5.9-5.8 Ma	16S rRNA	Yes	Cloning, qPCR	[27]

Table S5: Modifications made to manufactures instructions during the isolation of DNA with two different kits.

NucleoSpin® Soil	**FastDNA™ Kit for Soil**
The effect of both buffers (SL1 and SL2) provided with the kit was tested	Sample was homogenized by vortexing (2 × 1 min), instead of application of FastPrep Instrument (step 4)
The enhancer SX provided with the kit was not used (step 2 of the instruction)	DNA was eluted from NucleoSpin® Column with 50 µl of DES
DNA was eluted from NucleoSpin® Column with 50 µl of DNase/Pyrogen-Free Water (DES) instead of suggested buffer SE	