Supporting Information

Evolution of the plankton paleome in the Black Sea from the Deglacial to Anthropocene

Marco J.L. Coolen[1*], William Orsi[2], Cherel Balkema[1], Chris Quince[3], Keith Harris[3], Sean Sylva[1], Mariana Filipova-Marinova[4], Liviu Giosan[2]

[1] Marine Chemistry and Geochemistry Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA, [2] Geology and Geophysics Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA, [3] School of Engineering, Glasgow University, Glasgow, G128LT, United Kingdom, [4] Varna Regional Museum of History, 9000 Varna, Bulgaria.

MATERIAL AND METHODS

Sedimentary materials and age models.

The sedimentary ancient plankton DNA (fossil plankton DNA), geochemical, and molecular isotopic datasets presented in this study are stemming from the upper 185 cm of Giant Gravity Core GGC18. The upper 125 cm of the same GGC18 and same depth intervals (one-cm sampling resolution throughout Unit I, Unit II) was previously used for a fossil DNA and lipid biomarker survey to reconstruct the Holocene succession of haptophyte algae as sources of fossil alkenone lipid biomarkers (1), and to reconstruct viral infection of past *E. huxleyi* populations (2). For this study we included nine one-cm intervals from the lacustrine/low salinity Unit III-C1 section (9.4-11.4 ka cal BP) and nine one-cm intervals from the gray-green T section (7.5-9.4 ka cal BP; subsampled at one-cm resolution). Briefly, GGC18 was recovered from a water depth of 971m in the western basin of the Black Sea (42˚46.569"N:28˚40.647"E) during cruise AK06 on the R/V *Akademik* (Institute of Oceanology, Bulgarian Academy of Sciences; IOBAS) in September 2006. Radiocarbon dating of bulk organic matter (1) revealed that the top 8 cm was not recovered through gravity coring. However, undisturbed cores of top laminated sediments, including the top fluffy layer, were obtained at the same location using a MC800 multicorer (Ocean Instruments, San Diego, CA). The core length of the resulting multicore 19 (MC19) and GGC18 combined used for molecular isotopic and fossil DNA analysis was 193 cm. Detailed sampling procedures including precautions taken to prevent (cross) contamination with foreign

DNA can be found elsewhere (1). Calibrated radiocarbon dates from both cores have been used to derive the simplest age model (3). Briefly, a quadratic function of the corrected depth was used for the interval between 0 and 124 cm corrected depth: $Age = 0.1733*(corrected\ depth)^2 + 37.216*(corrected\ depth), \quad r^2 = 0.9958.$

Corrected depth refers to depth of samples in GGC18 to which 8 cm was added to compensate for the lost core top; no correction is applied to depths of samples from MC19. Sedimentation rates between 124 and 8,944 cal. years BP are low and, thus, interpolation between the calibrated radiocarbon dates corresponding to these depths was used to derive each sample age from that interval (3). For this study, the age model was extended into the deglacial with two dates on micro-gastropod shells. These radiocarbon dates were calibrated using the marine reservoir age based on the latest information that between 8000 and 11,500 years BP the Black Sea-Lake reservoir age was between 300 and 500 years (4). In addition, the archived core half was digitally photographed, subjected to X-radiography, and scanned for bulk elemental composition using an ITRAX^{TM} micro-XRF scanner as described previously (1). The XRF measurements were performed using a molybdenum x-ray tube at an exposure time of 10 sec per measurement and a step-size of 200 µm to allow for analysis of bulk chemistry at lamina and intra-lamina scales. The downcore variability in calcium was used herein to further characterize the core lithostratigraphy (4).

Lipid geochemistry and hydrogen isotope analysis (salinity proxy).

Total organic carbon (TOC) analysis, extraction of alkenones and compound identification from 21 freeze-dried one-cm sediment intervals of core GGC18 and MC19 compound identification and subsequent determination of *D/H* alkenone ratios from 21 sediment intervals aging between 6.6 and 0.8 ka cal BP has been described in detail elsewhere (1, 3).

Fossil plankton DNA

Extraction of sedimentary DNA

For this study, total DNA was extracted from 8-10 gram of wet weight sediment from the nine Unit III-C1 and nine Unit III-T one cm intervals using the PowerMax™ Soil DNA Isolation Kit (Mobio, Carlsbad, CA). The same approach was used previously to extract DNA at 1 cm resolution from the marine Unit I and II sediments of GGC18, which served for the reconstruction of haptophyte algae (1) and their associated viruses (2). The concentration of extracted DNA was quantified with the fluorescent dye PicoGreen (Molecular Probes). Genomic DNA extracted from all 136 sediment intervals spanning the last 11,400 years of deposition served as template for the targeted PCR/DGGE/capillary sequencing approach to reconstruct past copepod species as independent proxies for SSS changes. Genomic DNA of 48 out of 144 sediment intervals served as template for 18S rDNA amplicon sequencing (pyrotag sequencing) (5) using the Roche 454 GS FLX platform (6) to reconstruct the overall past plankton dynamics.

Fossil copepod 18S rDNA: group-specific PCR/DGGE/Sanger sequencing.

Partial copepod 18S rDNA (317 bp) was amplified with the general eukaryote primer EukA (7) and a novel copepod-specific reverse primer Cop317r (5'-TTG ATA GGG CAC ACC TTC-3'). Final primer concentrations were 0.25 µM. All reactions were performed in a Masterplex PCR cycler (Eppendorf). Each amplification reaction contained 0.25 mM of each deoxynucleotide (dNTP), 1.5 mM of $MgCl_2$, 50 µg of BSA (Ambion), 5 µl of 10 x PicoMaxx™ reaction buffer (Stratagene, LaJolla, CA), 2.5 units of DNA polymerase (PicoMaxx™ high fidelity PCR system; Stratagene), 0.2 µM of primers (Thermo-Fisher, Ulm, Germany), and 10 ng of DNA template. The reaction mixtures were adjusted to a final volume of 50 µl with nucleic acids and nuclease-free water (Ambion). Pipetting of the initial PCR mixtures were performed inside our ancient DNA-dedicated clean lab with all necessary precautions to prevent contamination of the reactions with foreign DNA as described recently (1). All PCR reactions also involved controls for contamination during pipetting of the reactions and for contamination during extraction and purification of DNA (1).

In order to minimize the formation of primer dimers, the copepod amplicons were generated in two steps. The first round of PCR involved a standard initial denaturing followed by 32 cycles including denaturing (30 s at 94ºC), primer annealing (60 s at 61.5ºC), and primer extension (60 s at 72ºC). A final extension was set to 10 min at 72ºC. The GC-clamp for subsequent DGGE analysis was incorporated by subjecting 1 µl of the amplicons ($\sim 10^7$ copies) to a second round of PCR with 12 cycles and 0.25 µM of primers EukA and GC-Cop317r (5'CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC CTT GAT AGG GCA CAC CTT C-3'). The underlined sequence in primer GC-Cop317r represents the 40-bp-long GC clamp, which is required to prevent complete melting of the amplicons during DGGE (8). The reverse primer for copepods was designed for this study based on aligned 18S rDNA sequences available through the SILVA database (9) and by using the Probe_Design tool implemented in the phylogenetic tree program ARB (10).

The above PCR products were separated based on genus to species-level variations in the nucleotide positions by DGGE (8). The polyacrylamide gels (6%, wt/vol) contained a denaturing gradient of 20 to 50% (with 100% denaturant equaling 7M urea and 40% formamide) for both copepod and haptophyte 18S rDNA. Gels were run for 15 h at 120 V (5 $V.cm^{-1}$) at 60ºC using a PhorU2 system (Ingeny, Leiden, Netherlands). DGGE bands were excised from the gel and prepared for subsequent cycle sequencing (1) using the facilities of Beckman Coulter Genomics. The partial copepod 18S rDNA sequences were automatically aligned against the SILVA release 111 eukaryote database (9), analyzed for the presence of chimeras, and unique OTUs were identified (97% sequence identity threshold) using MOTHUR (11). The most similar sequences of the seven resulting OTUs were identified through a BLAST (12) search in the National Center for Biotechnology Information non-redundant (NCBI nr) database (http://www.ncbi.nlm.nih.gov/).

Reconstruction of the overall eukaryotic plankton composition (pyrotag sequencing).

Partial (563 bp-long) environmental eukaryotic 18S rRNA gene fragments (V1V3 region) were amplified by PCR from the fossil DNA pool of the 48 selected sediment intervals spanning the last 11.4 kyr of the Black Sea's history. The same primer combinations (Table 1), additional PCR reagents, as well as PCR conditions to generate template DNA for subsequent multiplexed pyrotag sequencing (5) using Roche 454 titanium chemistry (6) were recently used to reconstruct the relative abundance of past dinoflagellates and diatoms in the GGC18 and MC19 samples (3). The concentration of each barcoded amplicon was determined using picogreen, and 600 nanograms of fifteen uniquely barcoded amplicons were pooled and purified as described recently (3). A total of 3 µg of purified template DNA (i.e., comprising a mixture of 15 barcoded amplicons at 200 ng per barcoded amplicon) was subjected to Roche 454 GS FLX Titanium pyrotag sequencing using the facilities of Engencore (Columbia, SC).

Primer name	1st PCR: with general eukaryote 18S rDNA primers, 5’ – 3’ orientation
EukA	ACC TGG TTG ATC CTG CCA GT
Euk516r	ACC AGA CTT GCC CTC C
	2nd PCR: with barcode 454 fusion forward primers (pyrosequencing template), 5’ – 3’ orientation
fpA_MID1_EukA	CGTATCGCCTCCCTCGCGCCATCAG**ACGAGTGCGT**ACCTGGTTGATCCTGCCAGT
fpA_MID2_EukA	CGTATCGCCTCCCTCGCGCCATCAG**ACGCTCGACA**ACCTGGTTGATCCTGCCAGT
fpA_MID3_EukA	CGTATCGCCTCCCTCGCGCCATCAG**AGACGCACTC**ACCTGGTTGATCCTGCCAGT
fpA_MID4_EukA	CGTATCGCCTCCCTCGCGCCATCAG**AGCACTGTAG**ACCTGGTTGATCCTGCCAGT
fpA_MID5_EukA	CGTATCGCCTCCCTCGCGCCATCAG**ATCAGACACG**ACCTGGTTGATCCTGCCAGT
fpA_MID6_EukA	CGTATCGCCTCCCTCGCGCCATCAG**ATATCGCGAG**ACCTGGTTGATCCTGCCAGT
fpA_MID7_EukA	CGTATCGCCTCCCTCGCGCCATCAG**CGTGTCTCTA**ACCTGGTTGATCCTGCCAGT
fpA_MID8_EukA	CGTATCGCCTCCCTCGCGCCATCAG**CTCGCGTGTC**ACCTGGTTGATCCTGCCAGT
fpA_MID10_EukA	CGTATCGCCTCCCTCGCGCCATCAG**TCTCTATGCG**ACCTGGTTGATCCTGCCAGT
fpA_MID11_EukA	CGTATCGCCTCCCTCGCGCCATCAG**TGATACGTCT**ACCTGGTTGATCCTGCCAGT
fpA_MID13_EukA	CGTATCGCCTCCCTCGCGCCATCAG**CATAGTAGTG**ACCTGGTTGATCCTGCCAGT
fpA_MID14_EukA	CGTATCGCCTCCCTCGCGCCATCAG**CGAGAGATAC**ACCTGGTTGATCCTGCCAGT
fpA_MID15_EukA	CGTATCGCCTCCCTCGCGCCATCAG**ATACGACGTA**ACCTGGTTGATCCTGCCAGT
fpA_MID16_EukA	CGTATCGCCTCCCTCGCGCCATCAG**TCACGTACTA**ACCTGGTTGATCCTGCCAGT
fpA_MID17_EukA	CGTATCGCCTCCCTCGCGCCATCAG**CGTCTAGTAC**ACCTGGTTGATCCTGCCAGT
	454 fusion reverse primer (5’-3’ orientation):
fpB_Euk516r	CTATGCGCCTTGCCAGCCCGCTCAGACCAGACTTGCCCTCC

Supporting Table S1. Primers used for Roche 454 pyrosequencing of PCR-amplified fossil 18S rRNA genes. fpA (454 fusion primer A sequence), MID (10-bp barcode sequence in **bold**), EukA (7) (forward primer sequence for general eukaryote 18S rDNA underlined). fpB (454 fusion primer B sequence), Euk516r (13) (reverse primer sequence for general eukaryote 18S rDNA).

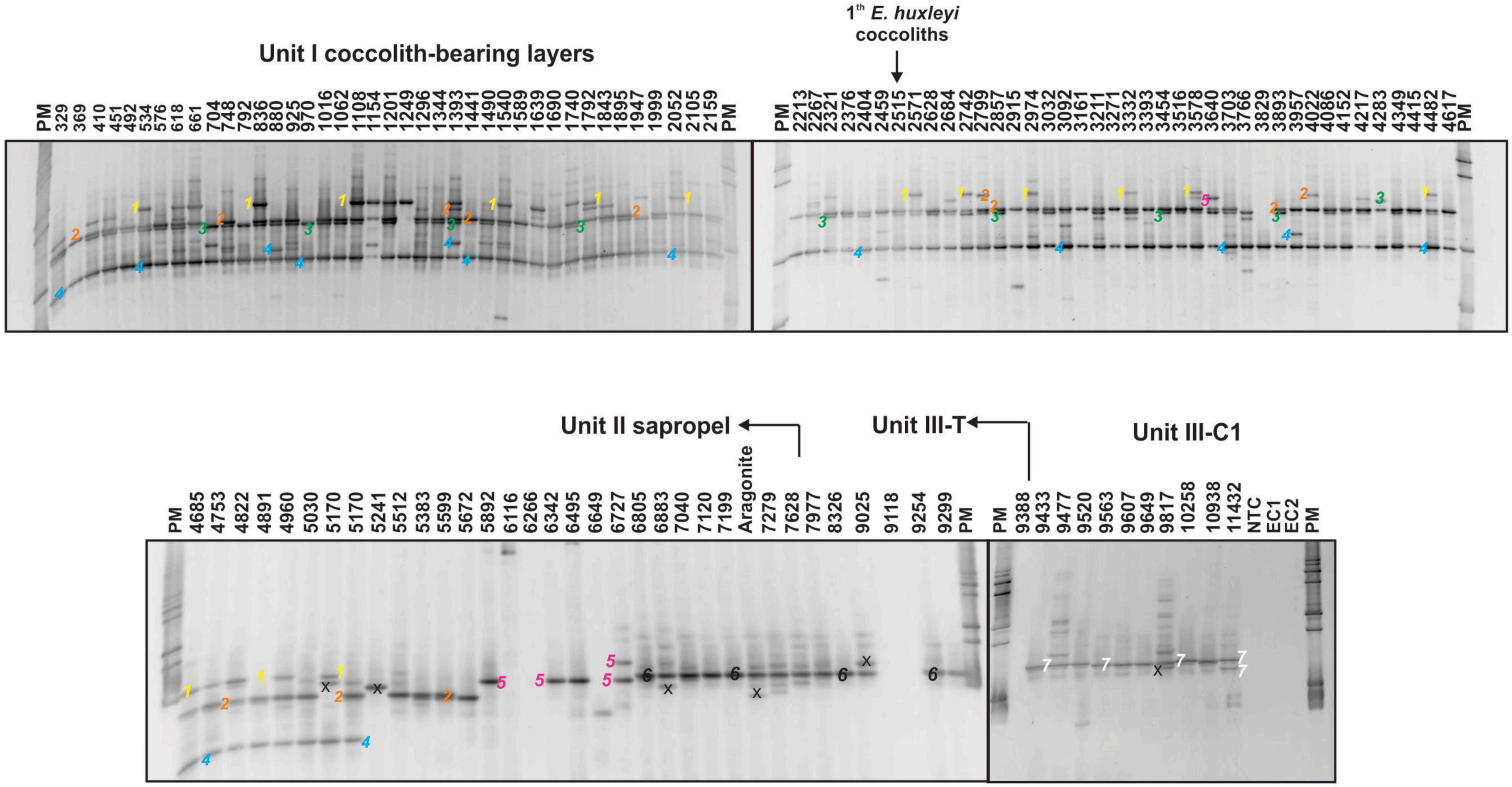
Pyrotag data processing and statistical analysis.

The corresponding fasta and qual files from the 454 pyrosequencing run and an associated mapping file that contained the names and corresponding barcode-primer sequences of the multiplexed samples were uploaded via the PyroTagger pipeline (http://pyrotagger.jgi-psf.org/) (14). Using Pyrotagger, reads were removed that, at a given read length, had >3% of bases with Phred values <27 (0.2% per-base error probability) to ensure that 97% clustering absorbed all erroneous reads. This user-defined length was in our case set to 360 bp (i.e., length-trimming), and shorter sequences were disregarded, followed by dereplication and clustering at 97% sequence identity threshold with the most abundant unique sequence serving as a cluster representative. Representative sequences from each cluster were classified by comparison to the SILVA database for eukaryotes (9). The current version of PyroTagger uses BLASTn (12) to identify the top hit and its associated taxonomy for each 97% cluster representative. Classified reads were separated into their respective samples using the barcodes provided in the original mapping file. Putative chimeric sequences were identified as having a best BLAST alignment <90% of the trimmed read length to the reference database, >90% sequence identity to the best BLAST match and cluster size ≤ 2.

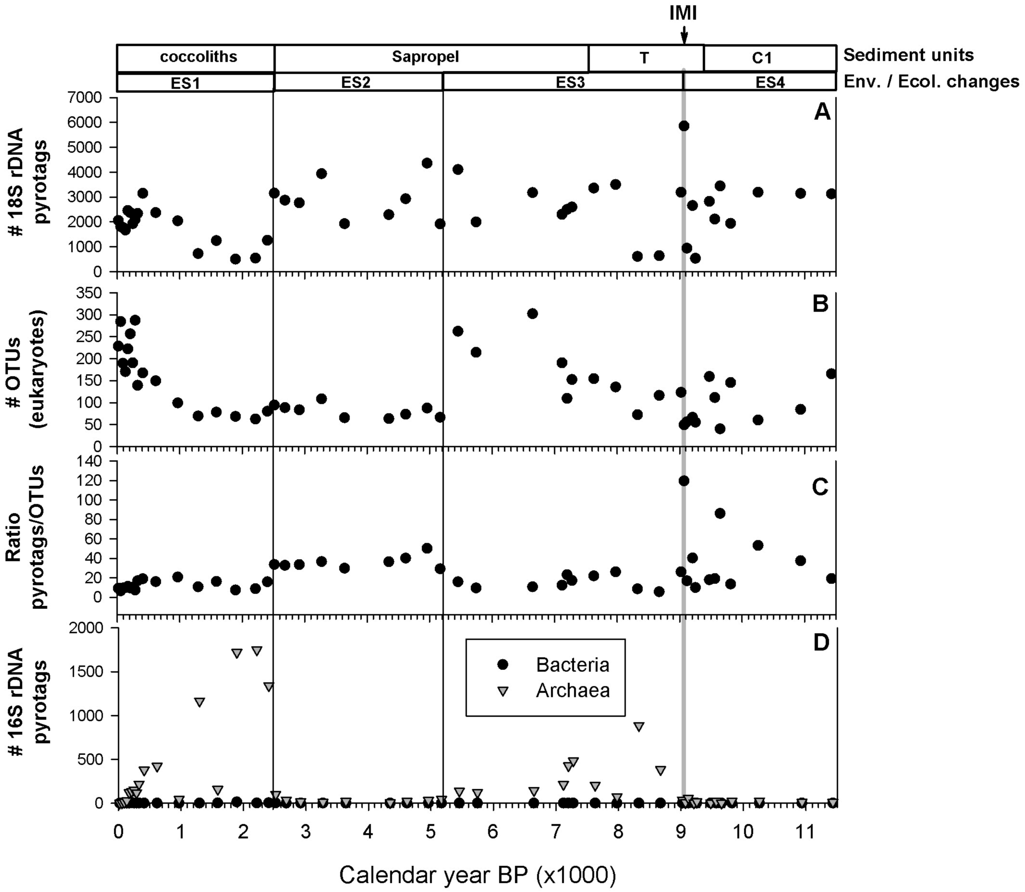
Non-metric multidimensional scaling (NMDS) and permutational multivariate analysis of variance were performed using the vegan package in R. In both cases the Bray-Curtis distance metrics was used to define dissimilarity between communities. The metaMDS function was used for NMDS, this employs multiple random starting configurations and selects the result with the lowest stress values. To determine the impact of the environmental stages on the community configurations, a permutational multivariate analysis of variance was performed using the adonis function. This partitions distance matrices between sources of variance and then uses a permutational approach to determine significance (15). The resulting ANOVA was judged highly significant with $P<0.001$ and 25.5% of the variance in the community structure explained by the environmental stage groupings.

Species (i.e., OTUs) with significant, nonrandom association with sediment samples were identified using the Indicator Species Analysis (ISA) tool in PC-ORD version 5.0. This analysis determines the constancy or faithfulness of species to a group by weighted averaging, which is the product of the relative abundance multiplied by relative frequency, times 100. In this way each species is given an importance value (IV), and indicator species for each group or cluster are those with the highest absolute value of IV (16). Significance was tested with a nonparametric procedure involving the Monte-Carlo permutation procedure in PC-ORD.

Neighbor-Joining bootstrap trees (1000 iterations) were made using the CLC Main Workbench 6.0 software package (CLC Bio, Cambridge, MA) for the majority of the 150 OTUs that according to ISA showed a significant response ($P<0.05$) to Deglacial and Holocene environmental changes in the Black Sea (i.e., those affiliated with cercozoans, chlorophytes, dinoflagellates, fungi, and stramenopiles). No trees were made for the few additional OTUs associated with other phyla.



Supporting Figure S1. DGGE analysis of PCR-amplified fossil Copepod 18S rDNA. In total, 69 DGGE bands were excised and sequenced. Bands melting at the same vertical position, and occasionally also bands at different vertical positions, represented identical sequences. As a result, the 69 bands comprised only seven unique (i.e., color coded) OTUs and are numbered 1-7 in the gel. A cross indicates low quality sequence. The calendar age in years BP of each analyzed interval is denoted above the gels as well as the different sediment types (Unit III-C1, Unit III-T, Unit II sapropel and Unit I coccolith-bearing layers). The first *E. huxleyi* coccoliths occurred at 2515 a cal BP. PM (position marker, not a sample). PCR controls: NTC (no template control; control for contamination during pipetting of the PCR reactions), EC1 and EC2 (Extraction Controls; control for contamination during extraction).



Supporting Figure S2. Distribution of SSU rDNA pyrotags and OTUs in the Black Sea record. (A) Number of high quality 18S rDNA pyrotags after PyroTagger analysis. (B) Number of eukaryotic OTUs in each sample. (C) Ratio between the number of 18S rDNA pyrotags and OTUs. (D) Number of bacterial and archaeal 16S rDNA OTUs in each sample. IMI: Previously published Initial Marine Inflow (17).

Clone DGGE_	NCBI closest Hit	Description	Source/habitat	E-value	Hit length	Identity	% Identity
Cop7	GU969142	*Sinocalanus sinensis*	River estuaries	1.78E-133	276	268	97.1
	GU969144	*Sinocalanus tenellus*	Brackish waters	4.34E-131	276	267	96.7
Cop6	AJ862482	Clone DGGE band 4DB81	Sulfurous freshwater lake	3.88E-122	248	243	98
	AY339*158-161*	*Skistodiaptomus* spp.	freshwater	2.47E-86	269	245	91
Cop5	GQ402489	clone 27_BSA6S5Euk	Black Sea surface waters	5.62E-152	275	275	100.0
	AY446901	*Centropages hamatus*	Black Sea	8.14E-145	274	273	99.6
Cop2	GU433130	clone BK316 18S	German North Sea	3.60E-153	277	277	100.0
	JF326205	*Paracalanus parvus*	Black Sea	4.32E-131	240	240	100.0
Cop4	GQ402478	clone 16_BSA6S5Euk	Black Sea suboxic zone	2.30E-154	279	279	100.0
	L81939	*Calanus pacificus*		2.30E-154	279	279	100.0
Cop1	AY331737	clone mj223	Bay of Fundy	1.84E-136	276	269	97.5
	GU969142	*Sinocalanus sinensis*	River estuaries	7.27E-136	276	269	97.5
Cop3	AY446903	*Pseudocalanus elongatus*	Black Sea	2.30E-154	279	279	100.0
	GQ402474	clone 12_BSA6S5Euk	Black Sea surface waters	2.30E-154	279	279	100.0

Supporting Table S2. Top GenBank hits and corresponding metadata for seven copepod sequences recovered from up to 11.4 kyr-old sediments of GGC18. See Fig 1*E* for the relative abundance of these copepods. Shown here are the most similar sequences (% identity) of unnamed environmental clones and named copepod species available through GenBank as well as the origin of the most similar clones or the typical habitat of the most similar named species.

Supporting Table S3 (pages 10-14). General information about the 150 OTUs, which showed a statistically significant ($p<0.05$) response to Deglacial and Holocene environmental changes in the Black Sea. The table shows the OTU numbers (e.g. Pyrotagger Cluster 5; C5), a description of the NCBI nr closest hits and corresponding accession numbers and e-values as well as broad taxonomic information and in which taxonomic tree (Figs. S5 to S9) the phylogenetic position with similar sequences from the NCBI nr database can be viewed. The table furthermore shows the most likely habitat and origin of the OTUs (F; freshwater M; marine/coastal, T; terrestrial) and information revealed from the Indicator Species Analysis (ISA): Percentage of perfect indication in each of the four identified environmental stages. A Monte Carlo test of significance of observed maximum indicator values for species (1000 permutations) revealed Observed Indicator Values (IV), and IV values from randomized groups (mean and standard deviation) as well as p-values. Environmentally relevant OTUs ($p<0.05$) are color-coded for comparison with data shown in Fig. 2*C* of the main manuscript and Figs. S5-S9 and are ranked for highest relevance (highest IV values) per environmental group.

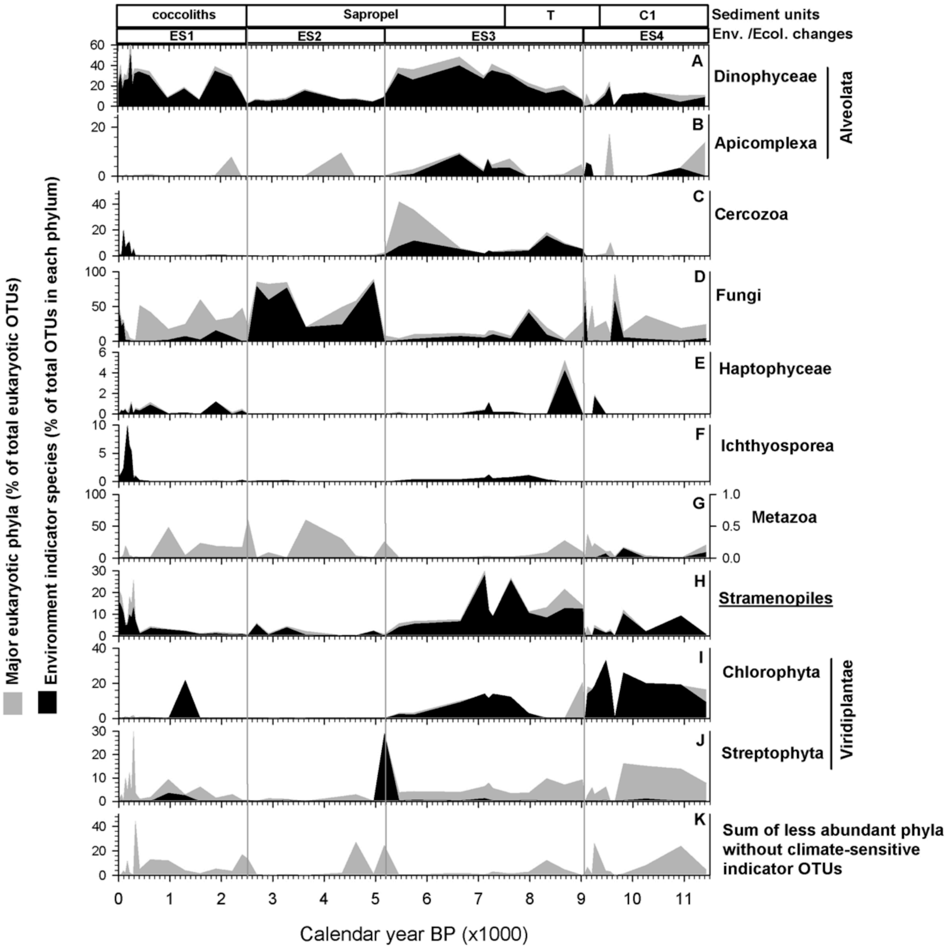
OTU	NCBI nr database closest hit (E-value)		Accession	E-value	Taxonomic /phylogenetic tree info		Most likely origin	(ES4) PFS	(ES3) DFS-IMS	(ES2) Most marine	(ES1) Freshening	(IV)	Mean	S.Dev	P-value
C5	*Choricystis* sp. Pic8/18P-11w	100.0	AY197629	1.00E-177	Viridiplantae	Chlorophyta	F	95	3	0	0	**94.6**	27.3	8.09	**0.0002**
C57	Uncult. freshwater eukaryote clone LG10-02	99.7	AY919716	5.00E-141	Alveolata	Dinophyceae	F	76	3	0	0	**76.3**	20.9	7.91	**0.0002**
C4429	*Choricystis* sp. Pic8/18P-11w	94.2	AY197629	2.00E-130	Viridiplantae	Chlorophyta	F	64	0	0	0	**63.6**	14.5	7.11	**0.0002**
C2766	*Chlorellaceae* sp. CCAP 283/3	99.1	FM205844	6.00E-165	Viridiplantae	Chlorophyta	F	62	0	0	0	**62.3**	15.3	7.52	**0.0002**
C19305	Uncult. freshwater eukaryote clone LG10-02	96.2	AY919716	3.00E-98	Alveolata	Dinophyceae	F	45	0	0	0	**45.5**	11.6	6.29	**0.002**
C8620	Uncult. freshwater eukaryote clone LG10-02	97.5	AY919716	2.00E-119	Alveolata	Dinophyceae	F	36	0	0	0	**36.4**	10.3	5.91	**0.0038**
C21790	Uncult. Chlorophyta clone 3-6	97.1	JF720727	3.00E-163	Viridiplantae	Chlorophyta	F	33	1	0	0	**32.9**	11.5	6.12	**0.0088**
C5470	Uncult. freshwater eukaryote clone LG10-02	95.6	AY919716	5.00E-101	Alveolata	Dinophyceae	F	31	3	0	0	**31.1**	12.3	6.33	**0.0156**
C6401	*Choricystis* sp. AS-29	97.3	AY195972	6.00E-160	Viridiplantae	Chlorophyta	F	27	0	0	0	**27.3**	9.4	5.67	**0.0226**
C11004	*Choricystis* sp. Pic8/18P-11w	96.2	AY197629	1.00E-156	Viridiplantae	Chlorophyta	F	27	0	0	0	**27.3**	9.7	5.54	**0.0208**
C24884	*Choricystis* sp. Pic8/18P-11w	95.6	AY197629	1.00E-152	Viridiplantae	Chlorophyta	F	27	0	0	0	**27.3**	9.6	5.51	**0.0226**
C2738	*Choricystis* sp. Pic8/18P-11w	95.0	AY197629	8.00E-139	Viridiplantae	Chlorophyta	F	27	0	0	0	**27.3**	10.6	5.59	**0.0244**
C10671	*Prasiola calophylla* isolate Pcal1	97.0	EF200521	8.00E-159	Viridiplantae	Chlorophyta	F	27	0	0	0	**27.3**	9.1	5.58	**0.0224**
C21809	Uncult. ascomycete clone 11-K17	99.1	EU333022	2.00E-159	Fungi	Dikarya	T	27	0	0	0	**27.3**	9.3	5.65	**0.0226**
C2649	Uncult. eukaryote clone BLF-37euk-AII-1-11d	94.1	GU970350	9.00E-109	Metazoa	Chordata	F	27	0	0	0	**27.3**	9.9	5.58	**0.0226**
C161	*Dreissena polymorpha*	96.4	AM774543	2.00E-130	Metazoa	Mollusca	F	27	0	0	0	**27.3**	9.3	5.67	**0.0226**
C19176	Uncult. eukaryote clone T08S1C3EukF5	97.1	JF829194	1.00E-132	Fungi	Dikarya	T	27	0	0	0	**27.3**	9.5	5.61	**0.022**
C4415	Uncult. eukaryote DGGE gel band ms_dino1_44	96.7	FJ797031	1.00E-156	Alveolata	Dinophyceae	M	27	0	0	0	**27.3**	10.6	5.62	**0.0248**
C31906	Uncult. freshwater eukaryote clone LG10-02	99.1	AY919716	1.00E-137	Alveolata	Dinophyceae	F	27	0	0	0	**27.3**	9.3	5.61	**0.0232**
C123	*Monocystis agilis*	93.2	AF457127	2.00E-115	Alveolata	Apicomplexa	M	27	0	0	0	**26.9**	10.6	5.98	**0.0272**
C147	*Prasiola meridionalis* isolate Pmer1	97.9	EF200528	2.00E-165	Viridiplantae	Chlorophyta	F	26	0	0	0	**26.1**	12.5	6.32	**0.0364**
C15266	Uncult. eukaryote DGGE gel band MS_euk3_34	97.9	FJ785950	4.00E-162	Viridiplantae	Streptophyta	T	25	1	0	0	**25**	11.1	6.2	**0.0266**
C990	*Choricystis* sp. Pic8/18P-11w	96.5	AY197629	6.00E-145	Viridiplantae	Chlorophyta	F	25	0	0	0	**24.9**	12.4	6.48	**0.0368**
C28992	*Choricystis* sp. AS-29	99.4	AY195972	1.00E-172	Viridiplantae	Chlorophyta	F	24	1	0	0	**24.3**	10.5	5.91	**0.0356**
C13559	Uncult. fungus DGGE gel band 6	97.6	FJ608611	5.00E-166	Fungi	Dikarya	T	23	0	0	1	**23**	10.1	5.62	**0.039**
C17	Uncult. marine clone BTPL20040810.0002	98.5	FJ222082	6.00E-150	Alveolata	Dinophyceae	M	0	87	1	2	**86.5**	34	8.87	**0.0002**
C32	Uncult. marine clone ANT-Roth-MECL-23	99.4	FJ985888	1.00E-142	Cercozoa		M	2	86	0	1	**86.1**	28.4	9.11	**0.0002**
C42	Uncult. eukaryote clone D4P08F09	92.7	EF100333	3.00E-24	Alveolata	Apicomplexa	M	1	80	0	0	**80.2**	21.1	8.57	**0.0002**
C68	Uncult. eukaryote clone CK06-06_EK293	99.2	AB521498	2.00E-115	Stramenopiles	Bolidophyceae	M	0	72	1	5	**72**	28.4	8.42	**0.0002**
C21	Uncult. eukaryote BS_DGGE_Euk-12a	99.7	DQ234292	2.00E-169	Alveolata	Dinophyceae	M	1	70	6	15	**69.6**	35.4	6.56	**0.0002**
C18	*Pseudopedinella elastica*	100.0	U14387	2.00E-160	Stramenopiles	Dictyochophyceae	M	6	69	2	22	**69.2**	42.5	7.54	**0.0002**

C28	Uncult. eukaryote clone PF1E3H04	100.0	HM226981	2.00E-124	Stramenopiles	Labyrinthulida	M	1	67	0	8	**66.6**	25.7	7.45	**0.0008**
C25	*Nannochloropsis* sp. Tow 2/24 P-1w	100.0	DQ977728	5.00E-176	Stramenopiles	Eustigmatophyceae	F	28	66	0	0	**65.8**	28.9	8.23	**0.0014**
C17974	*Gymnodinium microreticulatum*	98.5	AB265965	5.00E-171	Alveolata	Dinophyceae	M	0	64	1	2	**63.9**	19.7	7.09	**0.0004**
C372	Uncult. eukaryote DGGE gel band MS_euk3_05	98.2	FJ785969	1.00E-142	Cercozoa		M	0	64	4	0	**63.9**	21.9	7.95	**0.0004**
C3498	*Lepidodinium chlorophorum*	98.8	AY331681	1.00E-172	Alveolata	Dinophyceae	M	0	62	0	0	**61.7**	15	7.06	**0.0002**
C135	Uncult. cercozoan 18S rRNA gene, clone B12	100.0	FN263033	2.00E-144	Cercozoa		M	0	61	0	0	**60.5**	16.3	7.28	**0.0002**
C8158	*Gymnodinium catenatum* strain CCMP414	97.7	DQ779990	1.00E-166	Alveolata	Dinophyceae	M	0	60	0	1	**60.2**	24	9.55	**0.0022**
C19	*Woloszynskia cincta* strain Nam Seon Kang	100.0	FR690459	8.00E-144	Alveolata	Dinophyceae	M	7	59	6	25	**59.4**	33.6	5.14	**0.0002**
C7306	*Gymnodinium catenatum* strain CCMP414	98.5	DQ779990	5.00E-171	Alveolata	Dinophyceae	M	0	58	0	1	**58.1**	17.5	7.59	**0.0002**
C269	*Desmodesmus intermedius* CCAP 258/37	100.0	FR865702	1.00E-176	Viridiplantae	Chlorophyta	F	1	57	1	1	**57.2**	20.1	7.31	**0.001**
C112	*Gymnodinium* cf. nolleri	100.0	AB265963	5.00E-176	Alveolata	Dinophyceae	M	0	57	0	6	**57**	27.5	8.62	**0.0064**
C1002	Uncult. marine eukaryote clone SGB1-194	99.7	JN418980	1.00E-137	Cercozoa		M	3	56	0	0	**56.5**	17	7.25	**0.0004**
C13637	*Woloszynskia cincta* strain Nam Seon Kang	97.4	FR690459	7.00E-130	Alveolata	Dinophyceae	M	1	56	1	9	**56.1**	22.1	6.69	**0.001**
C149	Uncult. eukaryote BS_DGGE_Euk-4	100.0	DQ234284	6.00E-155	Cercozoa		M	0	56	4	1	**55.6**	28	9.77	**0.0148**
C173	Uncult. eukaryote clone RS1E5G05	98.8	HM228034	1.00E-136	unclassified		M	0	55	1	2	**54.6**	16.9	6.87	**0.0006**
C17429	*Gymnodinium catenatum* strain CCMP414	97.6	DQ779990	2.00E-165	Alveolata	Dinophyceae	M	0	53	0	0	**53.2**	16	7.61	**0.0006**
C22	*Cochliobolus cynodontis* strain NBRC 9793	100.0	JN941646	2.00E-179	Fungi	Dikarya	T	0	53	0	0	**53.1**	25.2	10.08	**0.0138**
C56	Uncult. eukaryote clone Pink_G08	93.6	GQ483808	6.00E-135	Alveolata	Dinophyceae	M	0	52	0	1	**51.9**	20.4	8.17	**0.0036**
C193	Uncult. eukaryote clone MS605-58	97.6	HM369676	1.00E-121	Cercozoa		M	0	52	2	9	**51.8**	30.7	9.43	**0.0304**
C1473	Uncult. eukaryote clone RF1E3A12	99.7	HM227701	4.00E-152	Cercozoa		M	0	52	6	1	**51.7**	17.1	6.52	**0.0006**
C151	Uncult. marine eukaryote clone EF_Dino_08	97.1	FJ210857	1.00E-161	Alveolata	Dinophyceae	M	1	51	0	2	**50.6**	22.1	8.3	**0.0082**
C113	Uncult. marine eukaryote isolate JPeuk-108	100.0	AY145115	1.00E-121	Fungi	Dikarya	T	0	48	0	9	**48.3**	20.7	7.83	**0.0068**
C24083	*Equiselum robustum*	96.8	X78890	2.00E-160	Viridiplantae	Streptophyta	T	8	46	0	0	**45.6**	17.3	7.47	**0.0054**
C1136	*Ophioglossum reticulatum*	99.1	AF313565	4.00E-162	Viridiplantae	Streptophyta	T	0	45	0	0	**45.5**	12.2	6.55	**0.0018**
C603	Uncult. marine eukaryote clone NPK2_119	98.5	EU371161	5.00E-146	Cercozoa		M	0	45	0	0	**44.5**	12.9	6.6	**0.0026**
C581	Uncult. marine picoeukaryote clone 1816H10	99.4	FR874836	7.00E-105	Alveolata	Dinophyceae	M	0	44	0	0	**44**	15.7	7.59	**0.0038**
C13	*Nannochloris eucaryota*	99.4	AB080304	5.00E-176	Viridiplantae	Chlorophyta	M	2	43	0	4	**42.7**	24.3	8.32	**0.0344**
C250	*Gregarina blattarum*	95.8	FJ459741	3.00E-44	Alveolata	Apicomplexa	M	1	39	0	2	**39.3**	18.5	7.77	**0.0204**
C610	*Gonyaulax cochlea* strain CCMP 1592	85.2	AF274258	7.00E-80	Alveolata	Dinophyceae	M	0	39	0	2	**39**	18.6	8.1	**0.0214**
C13489	*Enallax acutiformis*	97.9	AB037089	1.00E-162	Viridiplantae	Chlorophyta	U	0	36	0	0	**36.4**	10.1	5.81	**0.0044**
C6725	Uncult. eukaryote clone PF1EM2A12	95.0	HM227181	2.00E-139	Alveolata	Dinophyceae	M	0	36	0	0	**36.4**	10.2	5.87	**0.0046**
C6657	Uncult. eukaryote DGGE gel band MS_euk3_05	97.9	FJ785969	2.00E-149	Cercozoa		M	0	35	1	2	**35.5**	13.9	6.52	**0.0138**
C3449	*Gymnodinium microreticulatum*	95.0	AB265965	5.00E-151	Alveolata	Dinophyceae	M	0	35	1	3	**35.1**	16	7.09	**0.0196**
C3529	Uncult. eukaryote clone PS1E4D02	100.0	HM227361	4.00E-147	Cercozoa		M	0	33	0	9	**33.2**	16	6.66	**0.0246**
C8488	marine eukaryote clone BTPL20040810.0002	95.3	FJ222082	8.00E-139	Alveolata	Dinophyceae	M	0	33	0	1	**32.8**	11	5.78	**0.0056**
C3624	*Nannochloropsis* sp. Tow 2/24 P-1w	96.2	DQ977728	6.00E-155	Stramenopiles	Eustigmatophyceae	F	12	31	0	0	**31**	15.2	6.9	**0.0296**

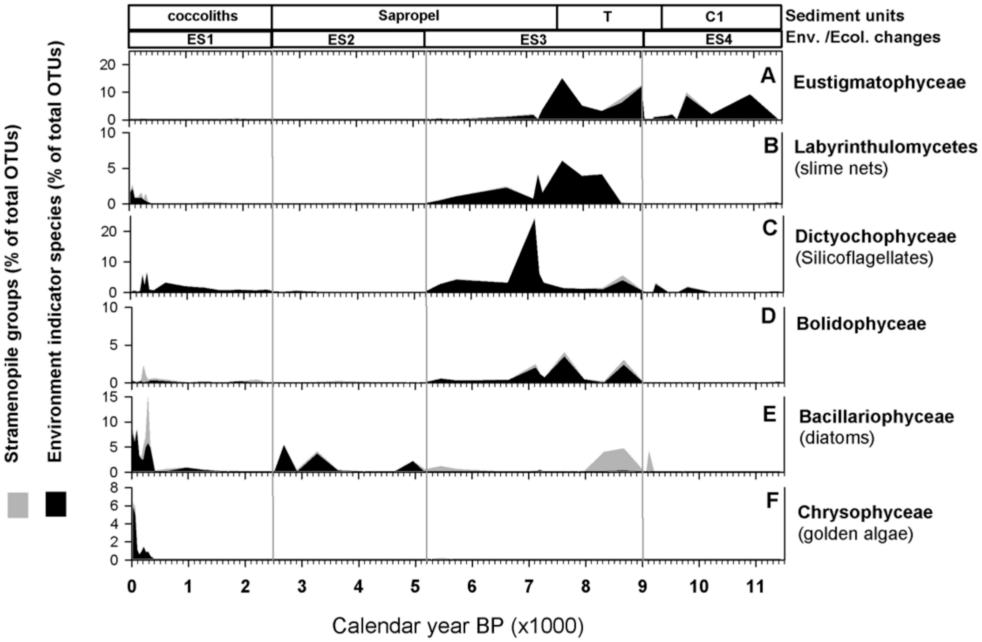
C8140	Uncult. eukaryote clone PF1EM2A12	96.8	HM227181	1.00E-142	Alveolata	Dinophyceae	M	0	30	0	1	**30.2**	11.1	5.75	**0.0118**
C23486	*Adiantum raddianum*	94.2	X78889	8.00E-134	Viridiplantae	Streptophyta	T	0	27	0	0	**27.3**	9.5	5.58	**0.0246**
C2984	*Gymnodinium* cf. nolleri	94.1	AB265963	1.00E-147	Alveolata	Dinophyceae	M	0	27	0	0	**27.3**	9.9	5.44	**0.021**
C17142	clone 8_BSA6S5Euk	94.1	GQ402470	2.00E-149	Alveolata	Dinophyceae	M	0	27	0	0	**27.3**	9.1	5.52	**0.0214**
C1438	Uncult. eukaryote clone Elev_18S_5171	92.7	EF025008	4.00E-97	Alveolata	Dinophyceae	M	0	27	0	0	**27.3**	9.6	5.61	**0.0246**
C27013	Uncult. eukaryote clone PF1EM2A12	93.3	HM227181	5.00E-101	Alveolata	Dinophyceae	M	0	27	0	0	**27.3**	10.1	5.44	**0.0194**
C5909	Uncult. eukaryote clone PF1EM2A12	94.9	HM227181	4.00E-87	Alveolata	Dinophyceae	M	0	27	0	0	**27.3**	9.6	5.62	**0.0238**
C5390	Uncult. eukaryote DGGE gel band ms_dino1_44	94.4	FJ797031	8.00E-144	Alveolata	Dinophyceae	M	0	27	0	0	**27.3**	9.2	5.59	**0.0218**
C9342	Uncult. eukaryote DGGE gel band MS_euk3_34	96.8	FJ785950	2.00E-159	Viridiplantae	Streptophyta	T	0	27	0	0	**27.3**	10.8	5.62	**0.0236**
C10657	Uncult. eukaryote clone 01RLS110600093	97.1	FN396512	5.00E-156	Viridiplantae	Streptophyta	T	0	27	0	0	**27.3**	9.1	5.57	**0.0236**
C12303	marine eukaryote clone BTPL20040810.0002	97.7	FJ222082	6.00E-145	Alveolata	Dinophyceae	M	0	27	0	0	**27.3**	10	5.54	**0.0212**
C9575	marine eukaryote clone BTQB20040603.0164	95.0	FJ221680	1.00E-147	Viridiplantae	Chlorophyta	M	0	27	0	0	**27.3**	9.2	5.54	**0.021**
C5088	*Warnowia* sp. BSL-2009a	93.5	FJ947040	2.00E-135	Alveolata	Dinophyceae	M	0	27	0	0	**27.3**	9.5	5.66	**0.0246**
C3088	Uncult. eukaryote clone CA-2-4-4h	97.6	HQ999313	2.00E-150	Viridiplantae	Streptophyta	T	0	26	0	0	**25.6**	11.8	6.21	**0.0358**
C20941	Uncult. eukaryote BS_DGGE_Euk-12a	97.6	DQ234292	2.00E-159	Alveolata	Dinophyceae	M	0	25	8	0	**25.3**	12.7	6.46	**0.0496**
C37488	*Gymnodinium catenatum* strain CCMP414	98.2	DQ779990	1.00E-167	Alveolata	Dinophyceae	M	0	24	1	0	**24.4**	11.8	6.27	**0.0412**
C3355	Uncult. alveolate clone PAG3AU2004	94.7	DQ244019	1.00E-136	Alveolata	Dinophyceae	M	0	24	0	1	**24.4**	10.8	5.85	**0.0372**
C14743	*Pseudopedinella elastica*	96.5	U14387	1.00E-142	Stramenopiles	Dictyochophyceae	M	0	24	0	1	**24.3**	10.7	5.87	**0.0382**
C4449	*Gyrodinium galatheanum* strain CCCM 555	92.7	AF274262	2.00E-130	Alveolata	Dinophyceae	F	0	24	0	1	**24.2**	10.3	5.72	**0.0326**
C4374	Uncult. eukaryote clone Pink_G08	92.2	GQ483808	2.00E-130	Alveolata	Dinophyceae	M	1	23	0	0	**23.3**	11.4	6.06	**0.0444**
C9849	marine eukaryote clone BTPL20040810.0002	95.6	FJ222082	6.00E-150	Alveolata	Dinophyceae	M	1	23	0	0	**23**	10	5.73	**0.0344**
C55	Uncult. eukaryote clone 01RLS110600093	99.4	FN396512	5.00E-171	Viridiplantae	Streptophyta	T	0	2	55	3	**54.8**	32.7	11.26	**0.0416**
C11	*Geomyces destructans* isolate MmyotGER-1	100.0	GU999983	5.00E-176	Fungi	Dikarya	T	6	11	54	14	**54.1**	33.4	6.87	**0.0096**
C1	*Tritarchium sp.* IAM 14522	100.0	AB003951	2.00E-179	Fungi	Dikarya	M	12	2	54	5	**53.6**	33.5	8.01	**0.02**
C17783	*Engyodontium album* strain IHEM4198	98.8	JF797223	4.00E-172	Fungi	Dikarya	M	0	0	53	2	**52.9**	15.4	6.68	**0.0008**
C19963	*Geomyces destructans* isolate MmyotGER-1	99.4	GU999983	1.00E-172	Fungi	Dikarya	T	1	3	47	3	**47.4**	21.2	7.64	**0.0086**
C4104	*Chamaeleomyces granulomatis* UAMH 11028	96.7	HM635076	1.00E-161	Fungi	Dikarya	T	0	0	47	8	**46.6**	17.3	7.08	**0.0042**
C2429	*Engyodontium album* strain IHEM4198	97.6	JF797223	3.00E-163	Fungi	Dikarya	T	0	0	39	5	**38.8**	13.6	6.27	**0.0052**
C29329	*Engyodontium album* strain IHEM4198	99.4	JF797223	5.00E-176	Fungi	Dikarya	T	0	1	38	1	**38**	12.2	6.29	**0.0052**
C3657	*Paecilomyces hepiali* strain SJL0909	99.7	HM135172	5.00E-171	Fungi	Dikarya	T	0	0	38	0	**37.5**	11.5	5.39	**0.0024**
C7461	DGGE gel band ms_dino1_35	94.2	FJ797027	1.00E-106	Alveolata	Dinophyceae	M	0	0	38	0	**37.5**	9.2	5.45	**0.0036**
C9712	*Westerdykella cylindrica* ATCC 24077	97.6	NG_016502	8.00E-164	Fungi	Dikarya	T	1	0	30	2	**29.7**	12.7	6.48	**0.0318**
C38835	*Geomyces destructans* isolate MmyotGER-1	98.8	GU999983	8.00E-169	Fungi	Dikarya	T	0	0	25	0	**25**	8.7	4.74	**0.0276**
C796	*Sancassania polyphyllae* clone MPJ-B6-9	97.9	JN166429	4.00E-157	Metazoa	Arthropoda	M	0	0	25	0	**25**	8.5	4.47	**0.021**
C21988	Uncult. eukaryote BS_DGGE_Euk-10	97.0	DQ234290	1.00E-132	Alveolata	Dinophyceae	M	0	0	25	0	**25**	8.6	4.52	**0.0224**

C18216	DGGE gel band ms_dino1_35	100.3	FJ797027	4.00E-97	Viridiplantae	Streptophyta	T	0	0	25	0	**25**	9	4.71	**0.0236**
C22131	Uncult. fungus DGGE gel band B4	97.3	JN020652	1.00E-161	Fungi	Dikarya	T	0	0	25	0	**25**	8.5	4.48	**0.021**
C11038	*Xenobotrytis acaducospora*	97.0	EU541483	1.00E-152	Fungi	Dikarya	T	0	0	21	1	**21.1**	9.1	5.5	**0.0398**
C2996	Uncult. marine eukaryote clone UEPACBp4	99.4	AY129045	3.00E-83	Alveolata	Dinophyceae	M	0	0	21	1	**21**	9.1	5.43	**0.0394**
C142	Uncult. eukaryote clone RS1E5F11	98.5	HM228078	1.00E-86	Alveolata	Dinophyceae	M	0	1	0	84	**84.4**	24.5	7.26	**0.0002**
C6823	Uncult. eukaryote clone PF1EM2A12	96.4	HM227181	8.00E-144	Alveolata	Dinophyceae	M	0	0	14	77	**77**	23.9	6.68	**0.0002**
C59	clone 20_BSA6S5Euk	100.0	GQ402482	4.00E-87	Alveolata	Dinophyceae	M	0	0	0	76	**76.2**	25.8	9.32	**0.0004**
C37	*Chaetoceros neogracile* strain AnM0002	87.0	EU090012	9.00E-104	Stramenopiles	Bacillariophyta	M	0	0	0	75	**75.5**	22	7.76	**0.0002**
C34	clone 19_BSA6S5Euk	100.0	GQ402481	2.00E-120	Unclassified		M	0	5	1	75	**74.6**	31.1	9.32	**0.0004**
C16	Uncult. eukaryote DGGE gel band MS_euk3_09	100.0	FJ785965	8.00E-129	Alveolata	Dinophyceae	M	0	1	12	63	**63.4**	27.5	7.77	**0.0008**
C94	Uncult. alveolate clone 29aD38	100.0	HM769621	2.00E-115	Alveolata	Dinophyceae	M	0	1	0	63	**63.1**	22.5	7.62	**0.0002**
C95	*Skeletonema grethae* CCAP 1077/4	100.0	FR865520	2.00E-179	Stramenopiles	Bacillariophyta	M	0	2	3	53	**52.9**	22.1	8.16	**0.0032**
C131	DGGE gel band ms_dino1_39	100.0	FJ797030	6.00E-160	Alveolata	Dinophyceae	M	0	8	10	52	**52.4**	24.5	7.06	**0.0042**
C5224	*Pentapharsodinium tyrrhenicum* GeoB*230	99.1	HQ845329	1.00E-172	Alveolata	Dinophyceae	M	0	4	0	52	**52.2**	18	6.7	**0.0014**
C49	*Scrippsiella trochoidea* strain CCMP2271	100.0	HM483396	5.00E-176	Alveolata	Dinophyceae	M	9	19	3	51	**50.6**	32.1	6.21	**0.0102**
C274	clone 25_BSA6S5Euk	100.0	GQ402487	4.00E-117	Alveolata	Dinophyceae	M	0	1	2	50	**49.8**	18.5	7.12	**0.0022**
C80	Uncult. stramenopile clone LC109.60	99.1	EU143916	4.00E-172	Stramenopiles	Chrysophyceae	T	0	0	0	49	**49.3**	16.5	7.36	**0.0022**
C146	Uncult. marine picoeukaryote clone 1816F11	100.0	FR874821	7.00E-115	Alveolata	Dinophyceae	M	0	3	0	49	**48.7**	17.4	6.78	**0.0024**
C20	DGGE gel band ms_dino1_35	100.0	FJ797027	4.00E-132	Alveolata	Dinophyceae	M	0	6	45	48	**47.7**	31.2	5.81	**0.0122**
C152	*Detonula confervacea* strain CCMP353	100.0	HQ912617	2.00E-179	Stramenopiles	Bacillariophyta	M	0	1	0	47	**46.8**	15.9	6.52	**0.0018**
C4305	*Peridinium wierzejskii*	97.1	AY443018	1.00E-162	Alveolata	Dinophyceae	F	0	3	1	46	**46.2**	17.6	6.42	**0.002**
C2800	Uncult. eukaryote clone JLRE-14-4	99.7	JF260907	2.00E-124	Alveolata	Dinophyceae	M	0	0	0	44	**44.4**	14.8	7.02	**0.0024**
C411	Uncult. eukaryotic picoplankton clone BK472	95.9	GU433155	2.00E-110	Alveolata	Dinophyceae	M	0	0	0	44	**44.4**	14.7	7.02	**0.0022**
C19548	clone SHAA470	96.2	JQ226412	8.00E-164	Alveolata	Dinophyceae	M	0	0	0	44	**44.4**	13.7	6.33	**0.0014**
C537	Uncult. stramenopile clone 3-E8	100.0	FN690609	3.00E-128	Stramenopiles	Bacillariophyta	M	0	0	0	43	**42.9**	15.5	7.13	**0.0044**
C347	*Chaetoceros* sp. ArM0005	100.0	EU090014	5.00E-126	Stramenopiles	Bacillariophyta	M	0	0	0	39	**38.9**	13	6.26	**0.004**
C1440	*Protoperidinium claudicans*	88.9	AB255833	5.00E-71	Alveolata	Dinophyceae	M	0	0	0	39	**38.9**	13	6.38	**0.0064**
C182	*Protoperidinium conicum*	92.9	AY443020	2.00E-71	Alveolata	Dinophyceae	M	0	0	0	39	**38.9**	14.3	7.24	**0.0098**
C914	Uncult. eukaryote clone BB01_97	99.4	AY885046	5.00E-116	Alveolata	Dinophyceae	M	0	0	0	39	**38.9**	12.7	6.13	**0.003**
C565	Uncult. eukaryote clone NAMAKO-15	96.4	AB252755	3.00E-103	Cercozoa		M	0	1	7	37	**37.3**	17.9	6.66	**0.015**
C18314	*Pentapharsodinium* sp. CCMP771	97.6	AF274270	5.00E-166	Alveolata	Dinophyceae	M	0	1	0	36	**35.6**	13.6	6.22	**0.009**
C10579	*Scrippsiella trochoidea* strain CCMP2271	99.1	HM483396	5.00E-171	Alveolata	Dinophyceae	M	0	8	2	34	**34.4**	18.6	6.82	**0.0326**
C1635	*Phellia exlex*	92.7	JF832984	1.00E-137	Fungi	Glomeromycota	T	0	0	0	33	**33.3**	12.2	6.1	**0.0076**
C1071	Uncult. dinoflagellate clone CCA32	98.8	AY179990	1.00E-131	Viridiplantae	Streptophyta	T	0	0	0	33	**33.3**	12.2	6.18	**0.007**
C44	Uncult. marine picoeukaryote clone 1804G02	97.0	FR874476	7.00E-120	Cercozoa		M	0	0	0	33	**33.3**	12.5	6.55	**0.012**

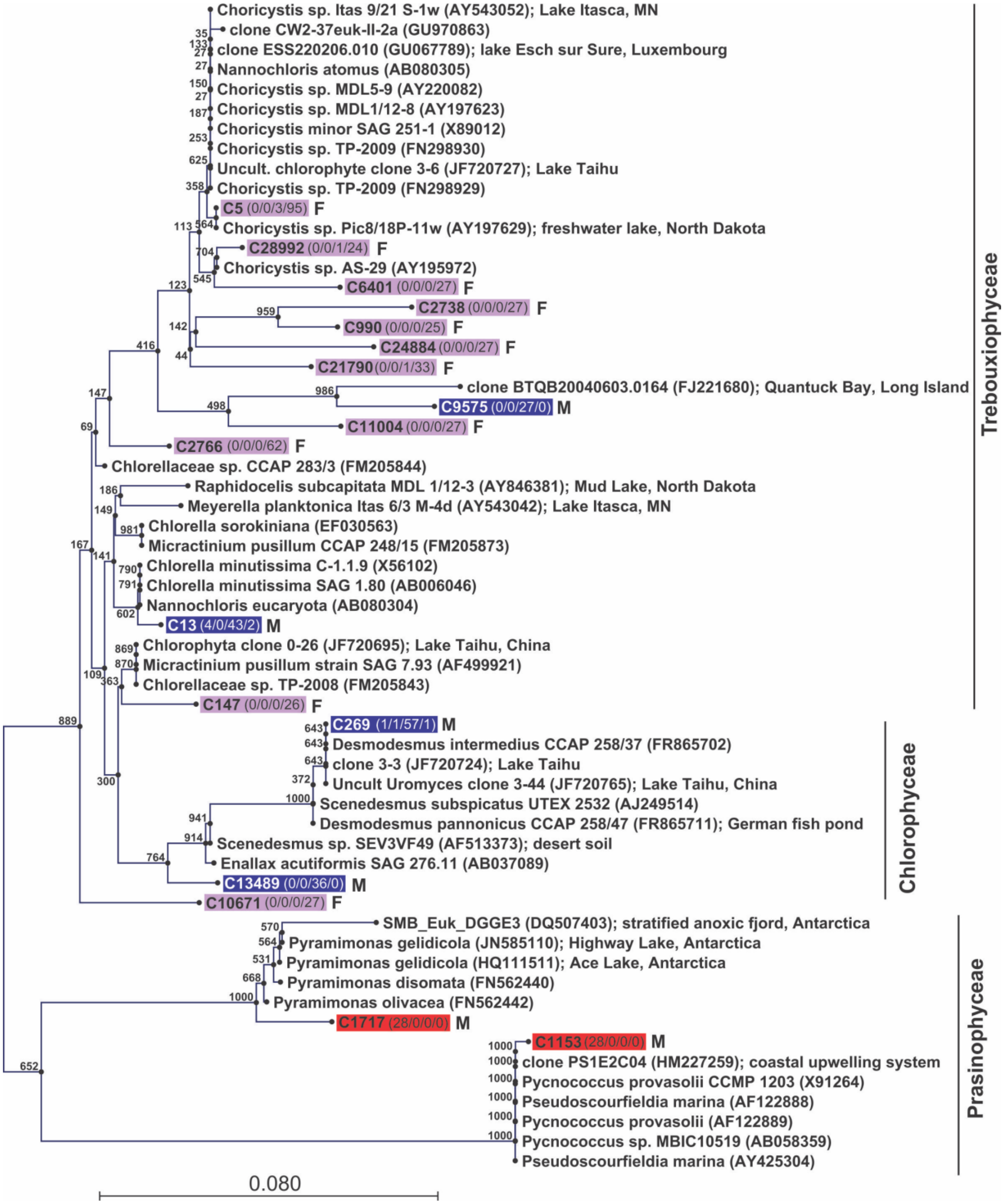
C2104	Uncult. eukaryote clone CS618-90	95.3	HM369645	1.00E-112	Alveolata	Dinophyceae	M	0	0	0	32	**32.4**	13	6.17	**0.0116**
C4148	Uncult. eukaryote DGGE gel band MS_euk3_09	97.1	FJ785965	2.00E-85	Alveolata	Dinophyceae	M	0	0	6	29	**29**	14.5	6.52	**0.0362**
C2197	*Alexandrium lusitanicum* strain AL-1	98.5	JF906999	1.00E-167	Alveolata	Dinophyceae	M	0	0	0	28	**27.8**	12	6.42	**0.0352**
C1369	*Calyptrosphaera* sp. LKM-2007-1	92.0	AM490992	4.00E-132	Haptophyceae	Coccolithales	M	0	0	0	28	**27.8**	11.1	5.88	**0.0314**
C6623	Dinophyceae sp. Shepherd's Crook	98.8	AY590479	6.00E-170	Alveolata	Dinophyceae	F	0	0	0	28	**27.8**	10.9	5.59	**0.0218**
C366	*Gonyaulax spinifera* CCAP 1118/2	88.5	FR865625	2.00E-95	Alveolata	Dinophyceae	M	0	0	0	28	**27.8**	11.9	6.38	**0.0288**
C1153	*Pycnococcus provasolii* CCAP 913/8	99.7	FR865764	4.00E-177	Viridiplantae	Chlorophyta	M	0	0	0	28	**27.8**	11.8	6.31	**0.0348**
C11448	*Scrippsiella trochoidea* strain CCMP2271	98.5	HM483396	2.00E-160	Alveolata	Dinophyceae	M	0	0	0	28	**27.8**	11.4	6.12	**0.0288**
C1829	Uncult. eukaryote clone BB01_97	95.0	AY885046	5.00E-116	Alveolata	Dinophyceae	M	0	0	0	28	**27.8**	11.4	6.06	**0.026**
C1912	Uncult. marine chrysophyte clone Biosope_T39.04	96.2	FJ537317	2.00E-129	Stramenopiles	Chrysophyceae	M	0	0	0	28	**27.8**	11.5	6.19	**0.0298**
C1717	*Pyramimonas olivaceae*	97.9	FN562442	1.00E-167	Viridiplantae	Chlorophyta	M	0	0	0	28	**27.8**	11	5.72	**0.0252**
C233	*Emiliania huxleyi*	100.0	FM882214	2.00E-179	Haptophyceae	Isochrysidales	M	0	10	3	27	**27.3**	10.3	5.56	**0.0222**
C2422	Uncult. marine cercozoan clone BIO2_A5	99.4	FN598236	5.00E-141	Cercozoa		M	0	0	0	27	**26.5**	12.3	6.17	**0.0416**



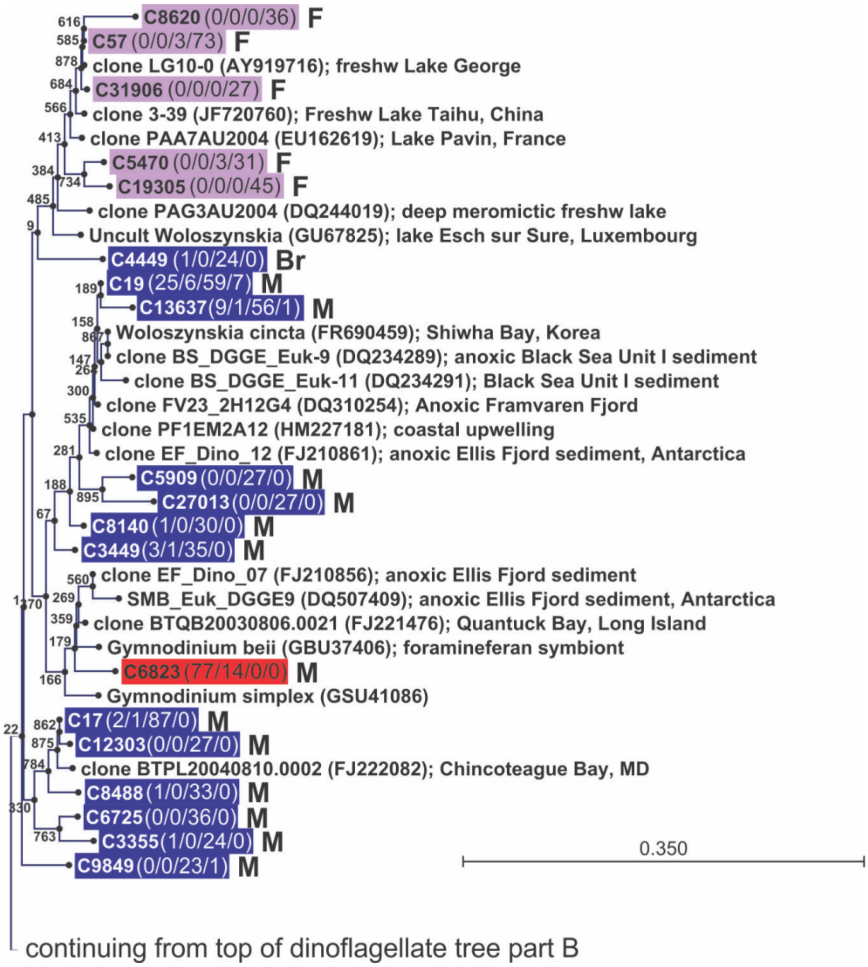
Supporting Figure S3. Normalized relative abundance of the predominant eukaryote phyla. The total percentage of pytotags grouping in the various phyla is shown in grey whereas the black surface areas represent the summed percentages of only statistically significant indicator species (i.e., from ISA analysis) for the different environmental stages. A higher taxonomic resolution of the underlined diverse stramenopiles is shown in Fig. S4.



Supporting Figure S4. Normalized relative abundance of the major stramenopile groups. The total percentage of pytotags for each stramenopile group is shown in grey whereas the black surface areas represent the summed percentages of only statistically significant indicator species (i.e., from ISA analysis) for the different environmental stages.

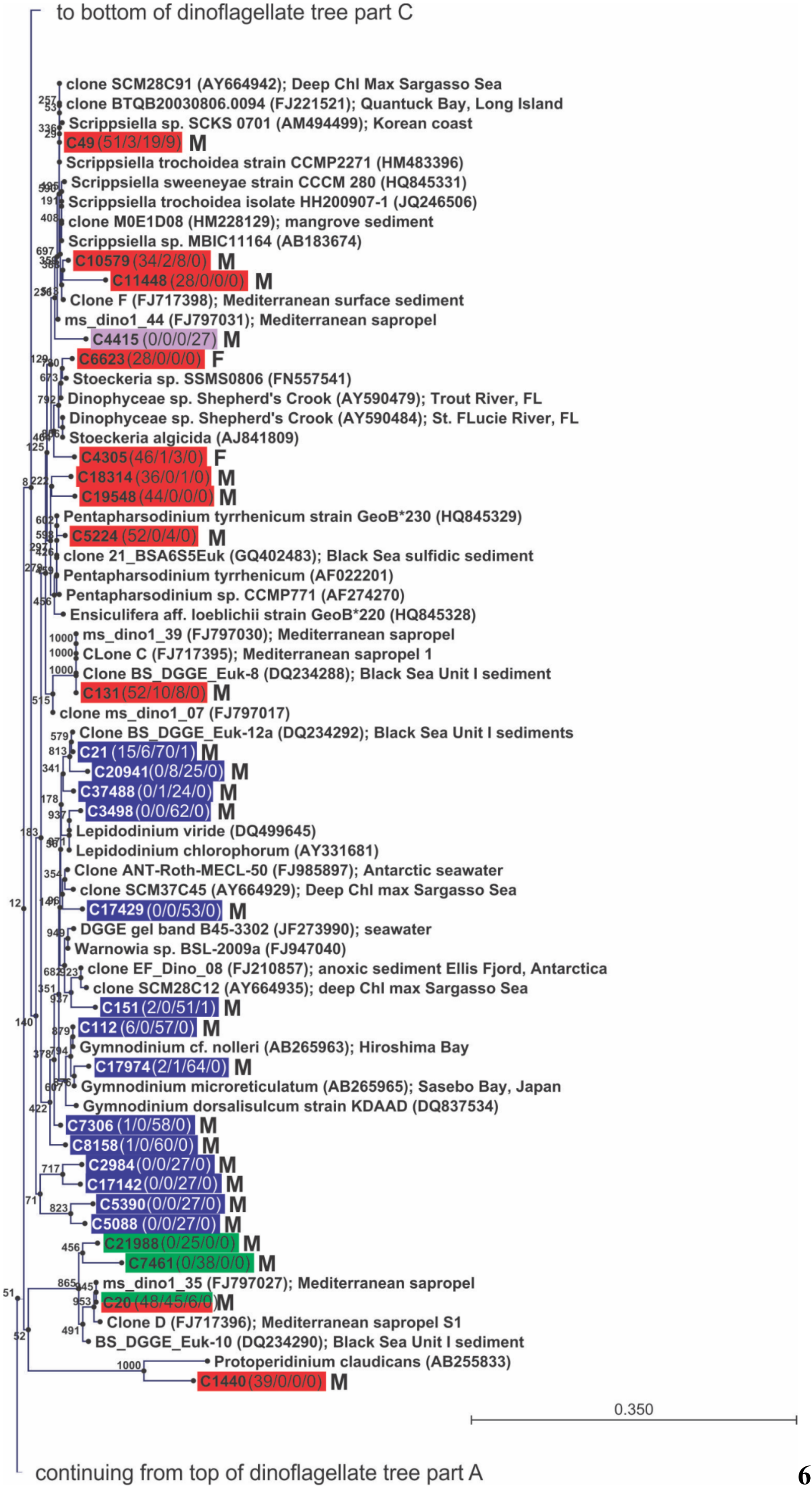


Supporting Figure S5. Neighbor Joining bootstrap tree with OTUs of past chlorophytes that represented statistically significant indicator species for the four environmental stages in the Black Sea as well as the most similar sequences available through GenBank. The percentage of perfect indication in ES 1-4 is depicted from Table S2 and listed in parenthesis next to each OTU. The maximum relevance of each OTU to an environmental stage is color coded as in Table S2. Listed is also the most likely origin of each OTU: F (freshwater), M (marine), T (terrestrial). The same strategy was used for tree Figs S6 to S9 and will therefore not be repeated in the figure captions below.



6A

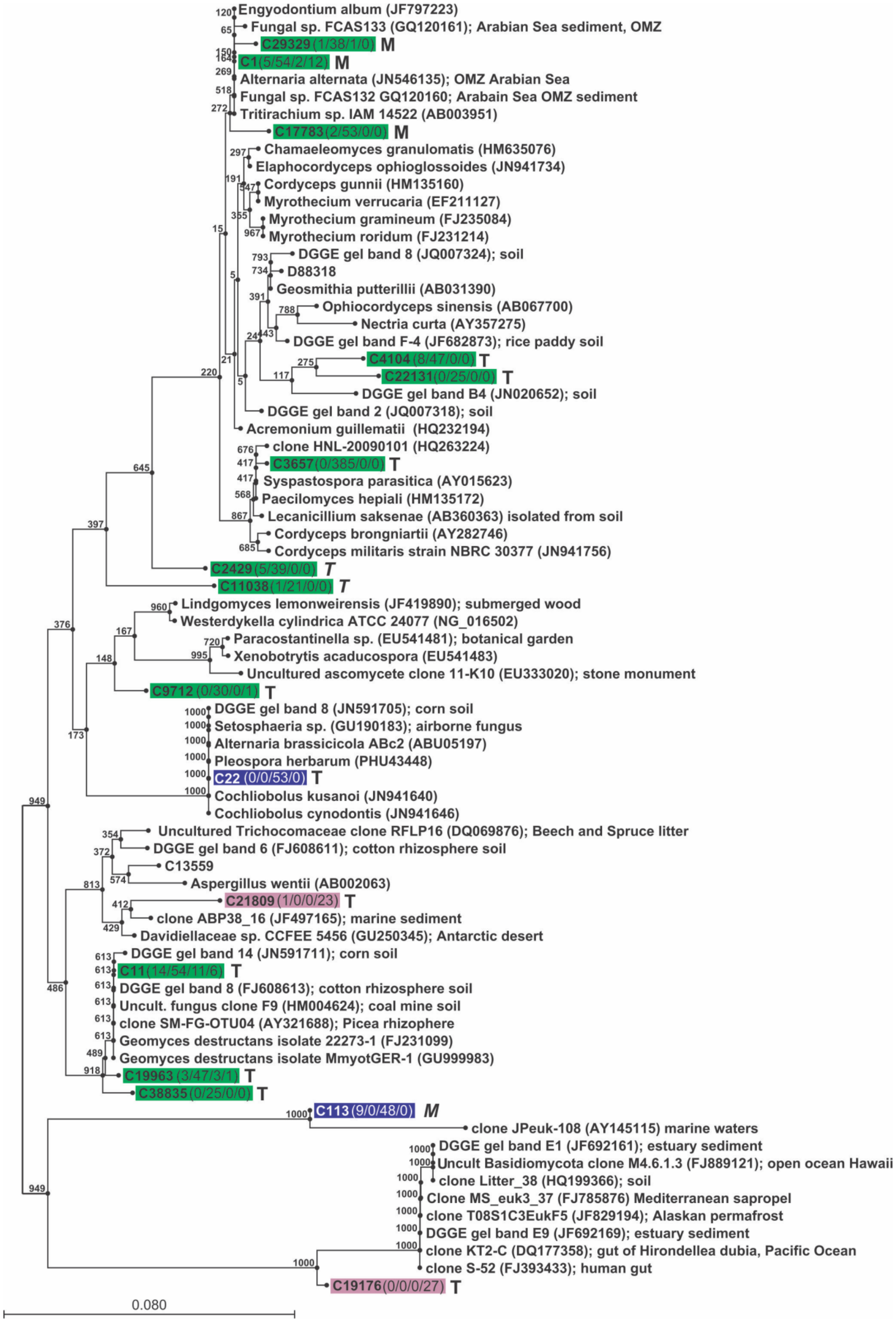
Supporting Figure S6 (pages 18-20). Neighbor Joining bootstrap tree with OTUs of past dinoflagellates that represented statistically significant indicator species for the four environmental stages in the Black Sea as well as the most similar sequences available through GenBank.


to bottom of dinoflagellate tree part C
clone SCM28C91 (AY664942); Deep Chl Max Sargasso Sea
clone BTQB20030806.0094 (FJ221521); Quantuck Bay, Long Island
Scrippsiella sp. SCKS 0701 (AM494499); Korean coast
C49 (51/3/19/9) M
Scrippsiella trochoidea strain CCMP2271 (HM483396)
Scrippsiella sweeneyae strain CCCM 280 (HQ845331)
Scrippsiella trochoidea isolate HH200907-1 (JQ246506)
clone M0E1D08 (HM228129); mangrove sediment
Scrippsiella sp. MBIC11164 (AB183674)
C10579 (34/2/8/0) M
C11448 (28/0/0/0) M
Clone F (FJ717398); Mediterranean surface sediment
ms_dino1_44 (FJ797031); Mediterranean sapropel
C4415 (0/0/0/27) M
C6623 (28/0/0/0) F
Stoeckeria sp. SSMS0806 (FN557541)
Dinophyceae sp. Shepherd's Crook (AY590479); Trout River, FL
Dinophyceae sp. Shepherd's Crook (AY590484); St. FLucie River, FL
Stoeckeria algicida (AJ841809)
C4305 (46/1/3/0) F
C18314 (36/0/1/0) M
C19548 (44/0/0/0) M
Pentapharsodinium tyrrhenicum strain GeoB*230 (HQ845329)
C5224 (52/0/4/0) M
clone 21_BSA6S5Euk (GQ402483); Black Sea sulfidic sediment
Pentapharsodinium tyrrhenicum (AF022201)
Pentapharsodinium sp. CCMP771 (AF274270)
Ensiculifera aff. loeblichii strain GeoB*220 (HQ845328)
ms_dino1_39 (FJ797030); Mediterranean sapropel
CLone C (FJ717395); Mediterranean sapropel 1
Clone BS_DGGE_Euk-8 (DQ234288); Black Sea Unit I sediment
C131 (52/10/8/0) M
clone ms_dino1_07 (FJ797017)
Clone BS_DGGE_Euk-12a (DQ234292); Black Sea Unit I sediments
C21 (15/6/70/1) M
C20941 (0/8/25/0) M
C37488 (0/1/24/0) M
C3498 (0/0/62/0) M
Lepidodinium viride (DQ499645)
Lepidodinium chlorophorum (AY331681)
Clone ANT-Roth-MECL-50 (FJ985897); Antarctic seawater
clone SCM37C45 (AY664929); Deep Chl max Sargasso Sea
C17429 (0/0/53/0) M
DGGE gel band B45-3302 (JF273990); seawater
Warnowia sp. BSL-2009a (FJ947040)
clone EF_Dino_08 (FJ210857); anoxic sediment Ellis Fjord, Antarctica
clone SCM28C12 (AY664935); deep Chl max Sargasso Sea
C151 (2/0/51/1) M
C112 (6/0/57/0) M
Gymnodinium cf. nolleri (AB265963); Hiroshima Bay
C17974 (2/1/64/0) M
Gymnodinium microreticulatum (AB265965); Sasebo Bay, Japan
Gymnodinium dorsalisulcum strain KDAAD (DQ837534)
C7306 (1/0/58/0) M
C8158 (1/0/60/0) M
C2984 (0/0/27/0) M
C17142 (0/0/27/0) M
C5390 (0/0/27/0) M
C5088 (0/0/27/0) M
C21988 (0/25/0/0) M
C7461 (0/38/0/0) M
ms_dino1_35 (FJ797027); Mediterranean sapropel
C20 (48/45/6/0) M
Clone D (FJ717396); Mediterranean sapropel S1
BS_DGGE_Euk-10 (DQ234290); Black Sea Unit I sediment
Protoperidinium claudicans (AB255833)
C1440 (39/0/0/0) M
257
53
336
29
408
697
191
254
129
673
792
464
125
8
602
598
297
279
456
1000
1000
1000
515
579
813
341
178
937
183
354
12
949
682
351
937
140
879
378
607
422
717
71
823
456
865
953
491
51
52
1000
0.350
continuing from top of dinoflagellate tree part A

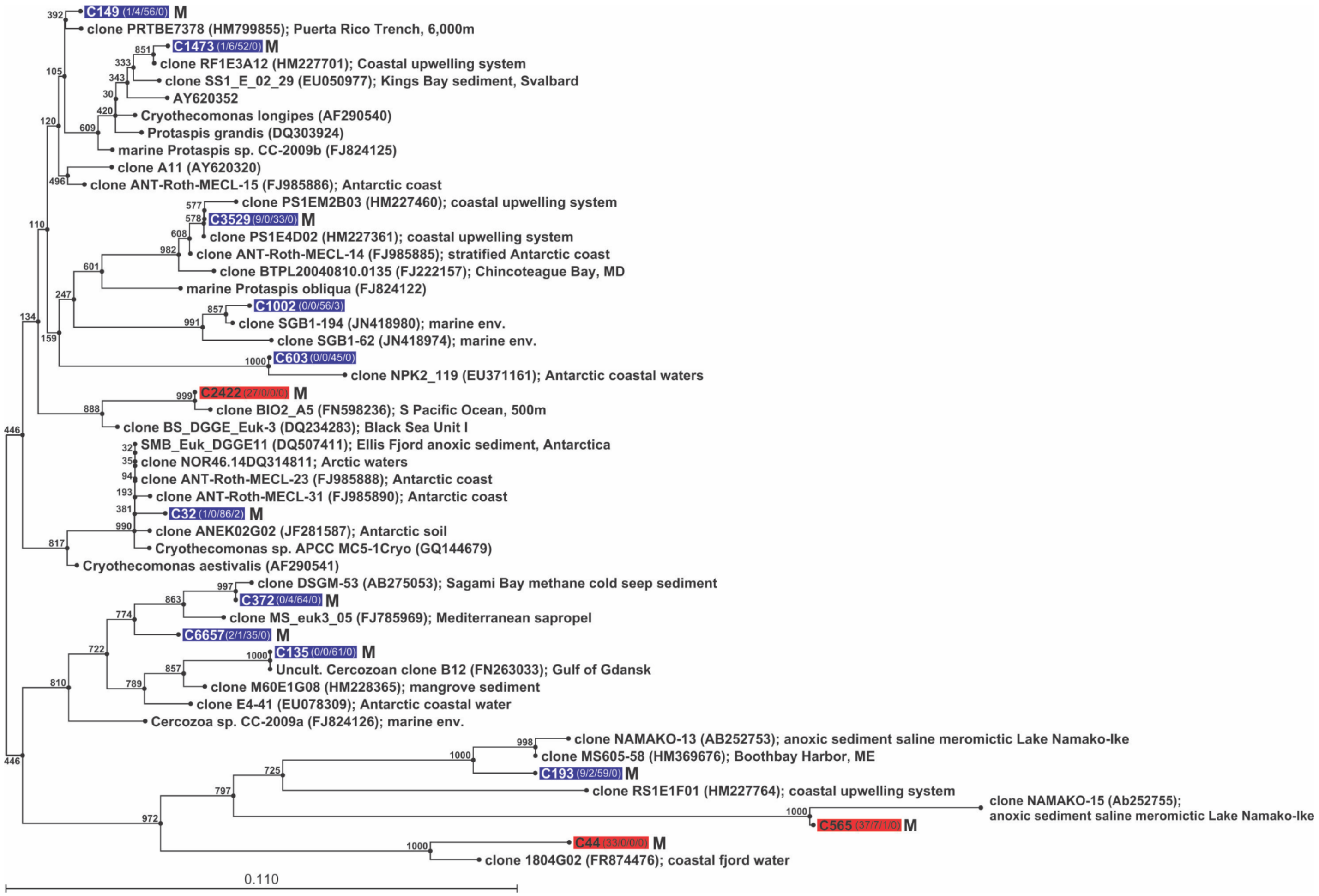
6B


to bottom of dinoflagellate tree part B
993
378
C56 (1/0/52/0) M
C4374 (0/0/23/1) M
clone Pink_G08 (GQ483808); intertidal thrombolite
543
clone MS605-10 (HM369667); Boothbay Harbor
869
clone 1816C12 (FR874791); fjord
521
clone 1816H10 (FR874836); fjord
552
C581 (0/0/44/0) M
1000
clone BTQB20030503.0083 (FJ221448); Quantuck Bay, Long Island
815
clone AD6S.30 (DQ647544); seawater
Pentapharsodinium tyrrhenicum clone 1501 (JF790993); Gulf of Mexico
342
781
clone RS1E5F11 (HM228078); coastal upwelling system
987
1000
clone RS1E4D08 (HM227952); coastal upwelling system
C142 (84/0/1/0) M
735
985
clone JJ18S_16 (GQ899153); seawater
Amoebophrya sp. ex Gymnodinium instriatum (AF472554); Chesapeake Bay
9
1000
clone UEPACBp4 (AY129045); Pacific Ocean
C2996 (1/21/0/0) M
91
1000
clone Elev_18S_5171 (EF025008); Soil rhizosphere
C1438 (0/0/27/0) T
648
clone SA18S_19 (GU474398); Nakdong River
869
clone BK472 (GU433155); North Sea near Helgoland
268
711
clone 1801G01 (FR874327); coastal fjord
C411 (44/0/0/0) M
970
993
Clone MS_euk3_09 (FJ785965); MEditerranean sapropels
1000
BS_DGGE_Euk-2 (DQ234282); Black Sea Unit I sediment
993
C16 (63/12/1/0) M
C4148 (29/6/0/0) M
1000
C146 (49/0/3/0) M
1000
clone 1816F11 (FR874821); fjord water
35
1000
clone FV36_CilD10 (DQ310305); Anoxic Mariager Fjord
1000
clone SCM16C10 (AY665019); Deep Chl max Sargaso Sea
clone NS51B231 (AJ829829); South China Sea
981
clone 20_BSA6S5Euk (GQ402482); Black Sea Unit I sediment
981
BS_DGGE_Euk-6 (DQ234286); Black Sea Unit I sediment
999
C59 (76/0/0/0) M
884
clone UEPACQp5 (AY129056); coastal Pacific
C2104 (32/0/0/0) M
606
clone JLRE-15-1 (JF260909); saltwater intruded river
606
clone 26_BSA6S5Euk (GQ402488); anoxic Black Sea surface sediment
689
606
clone NA2_4G2 (EF526806); Framvaren Fjord
430
606
lone M3_18E07 (DQ103803); anoxic Mariager Fjord
606
clone 29aD38 (HM769621); South China Sea
606
lone cs618-79 (HM369634); Boothbay Harbor, ME
606
clone M2_18F03 (DQ103797); anoxic Mariager Fjord
879
C94 (63/0/1/0) M
1000
clone CS618-90 (HM369645); Boothbay Harbor, ME
930
clone JLRE-14-4 (JF260907); saltwater intruded river
1000
497
C2800 (44/0/0/0) M
clone 25_BSA6S5Euk (GQ402487); Black Sea sulfidic surface sediment
623
998
C274 (50/2/1/0) M
Euduboscquella sp. ex Favella arcuata (JN934989)
Euduboscquella sp. ex Tintinnopsis cf. subacuta (JN934992)
1000
Gonyaulax cochlea strain CCMP 1592 (AF274258)
341
C610 (2/0/39/0) M
1000
clone D4P08F09 (EF100333); anoxic marine surface sediment
143
C42 (0/0/80/1) M
869
Gonyaulax spinifera (FR865625)
C366 (28/0/0/0) M
64
995
C182 (39/0/0/0) M
1000
Protoperidinium conicum (AY443020)
Protoperidinium conicum (AB181887); seawater
38
611
Alexandrium sp. tamutum strain SZN29 (AJ535378)
157
Alexandrium ostenfeldii (AB538439); Funka Bay, Japan
Alexandrium insuetum strain AI104 (AB088298)
1000
536
Alexandrium minutum strain AM (JF906998)
343
Alexandrium minutum strain SZN30 (AJ535380)
405
Alexandrium minutum strain CCMP 113 (AY831408)
Alexandrium minutum strain AL3T (AJ535388)
51
296
C2197 (28/0/0/0) M
999
C914 (39/0/0/0) M
845
clone BB01_97 (AY885046); estuary, Barnegat Bay, NJ
1000
eukaryote clone M3_18B06 (DQ103863); anoxic Mariager Fjord
C1829 (28/0/0/0) M
0.350

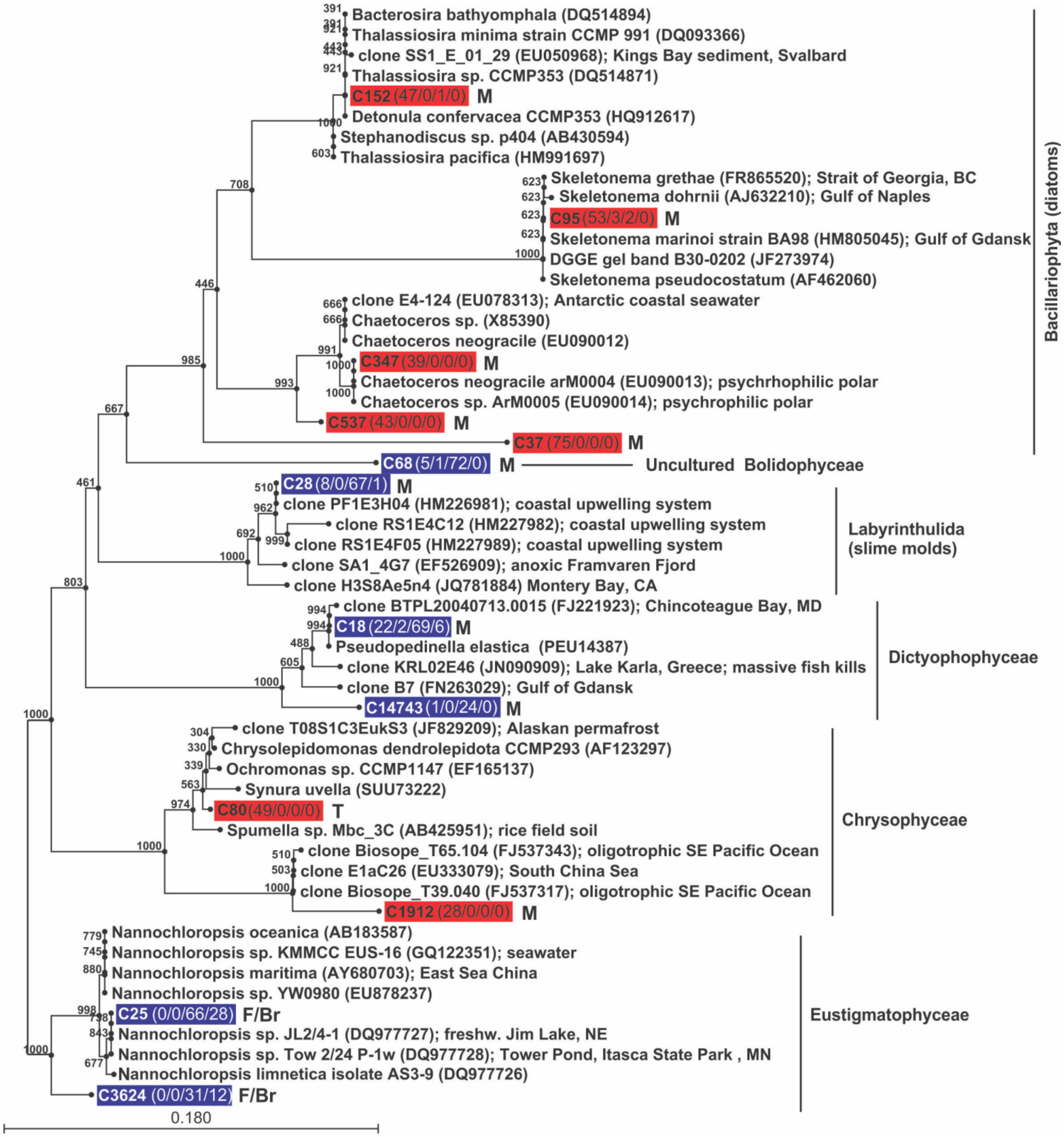
6C



Supporting Figure S7. Neighbor Joining bootstrap tree with OTUs of fungi that represented statistically significant indicator species for the four environmental stages in the Black Sea as well as the most similar sequences available through GenBank.



Supporting Figure S8. Neighbor Joining bootstrap tree with OTUs of past cercozoa that represented statistically significant indicator species for the four environmental stages in the Black Sea as well as the most similar sequences available through GenBank.



Supporting Figure S9. Neighbor joining bootstrap tree with OTUs of phototrophic (Bacillariophyta, Bolidophyceae, Dictyochophyceae, Crysophyceae, Eustigmatophyceae) and heterotrophic (Labyrinthylida) stramenopiles that represented statistically significant indicator species for the four environmental stages in the Black Sea as well as the most similar sequences available through GenBank.

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