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Morphological and genetic diversity of Beaufort Sea diatoms with high contributions from the *Chaetoceros neogracilis* species complex

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1 **Morphological and genetic diversity of Beaufort Sea**
2 **diatoms with high contributions from the *Chaetoceros***
3 ***neogracilis* species complex**

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29 **Abstract**

30

31 Seventy-five diatoms strains isolated from the Beaufort Sea (Canadian Arctic) in the summer of
32 2009 were characterized by light and electron microscopy (SEM and TEM) as well as 18S and 28S
33 rRNA gene sequencing. These strains group into 20 genotypes and 17 morphotypes and are
34 affiliated with the genera *Arcocellulus*, *Attheya*, *Chaetoceros*, *Cylindrotheca*, *Eucampia*, *Nitzschia*,
35 *Porosira*, *Pseudo-nitzschia*, *Shionodiscus*, *Thalassiosira*, *Synedropsis*. Most of the species have a
36 distribution confined to the northern/polar area. *Chaetoceros neogracilis* and *Chaetoceros gelidus*
37 were the most represented taxa. Strains of *C. neogracilis* were morphologically similar and shared
38 identical 18S rRNA gene sequences, but belonged to four distinct genetic clades based on 28S
39 rRNA, ITS-1 and ITS-2 phylogenies. Secondary structure prediction revealed that these four clades
40 differ in hemi-compensatory base changes (HCBCs) in paired positions of the ITS-2, suggesting
41 their inability to interbreed. Reproductively isolated *C. neogracilis* genotypes can thus co-occur in
42 summer phytoplankton communities in the Beaufort Sea. *Chaetoceros neogracilis* generally
43 occurred as single cells but can also form short colonies. It is phylogenetically distinct from an
44 Antarctic species, erroneously identified in some previous studies as *C. neogracilis* but named here
45 as *Chaetoceros* sp. This work provides taxonomically validated sequences for 20 Arctic diatom
46 taxa, which will facilitate future metabarcoding studies on phytoplankton in this region.

47

48 **Key index words:** biogeography, ITS, ITS2 secondary structure, LSU, morphology, phylogeny,
49 polar diatoms, SSU

50 **Abbreviations:** CCMP, National Centre for Marine Algae and Microbiota; DCM, Deep Chlorophyll
51 Maximum; ITS, Internal Transcribed Spacer; ITS-1, first internal transcribed spacer; ITS-2,
52 second internal transcribed spacer; RCC, Roscoff Culture Collection; T-RFLP, terminal-RFLP;
53

54 INTRODUCTION

55

56 Due to fluctuations in light, temperature, salinity and sea ice extent, Arctic phytoplankton
57 undergo high seasonal variability in abundance and composition. Higher temperatures and longer
58 daylight between March and September, lead to an increase in algal biomass and primary
59 production (Sherr et al. 2003, Wang et al. 2005). Diatoms account for a high portion of Arctic
60 phytoplankton, especially in coastal locations (Booth & Horner 1997, Lovejoy et al. 2002) and
61 species belonging to the genera *Chaetoceros* Ehrenberg and *Thalassiosira* Cleve can dominate
62 phytoplankton communities in different regions (Tuschling et al. 2000, Booth et al. 2002, Ratkova
63 & Wassmann 2002).

64 The Beaufort Sea is a major component of the Arctic Ocean, and is highly influenced by the
65 Mackenzie River, which plays a key role in disrupting the winter ice in early spring promoting
66 primary production and phytoplankton blooms (Carmack & MacDonald 2002). In addition, periodic
67 wind-driven upwelling events can bring nutrient rich waters up to the surface layer and promote
68 phytoplankton growth (Pickart et al. 2013). Except during episodic upwelling events, the water
69 column is highly stratified, the nutrient concentration in the upper layers is extremely low, leading
70 to the prevalence of picoeukaryotes, mostly represented by the psychrophilic *Micromonas* Manton
71 & Parke ecotype corresponding to the single genetic clade named “Arctic *Micromonas*” (Lovejoy et
72 al. 2007, Balzano et al. 2012b), within the phytoplankton community. Diatoms tend to be more
73 abundant near the coast (Hill et al. 2005), occasionally blooming in late spring (Hill et al. 2005,
74 Sukhanova et al. 2009). The algal biomass and the contribution of diatoms to the phytoplankton
75 community increase in summer (Hill et al. 2005) and diatoms bloom more frequently at the deep
76 chlorophyll maximum (DCM; Sukhanova et al. 2009). Autumn communities include higher
77 contributions of dinoflagellates, which can dominate the community along with diatoms (Brugel et
78 al. 2009).

79 The MALINA oceanographic expedition sailed in July 2009 from the Pacific coast of Canada to
80 the Beaufort Sea where an extensive multidisciplinary sampling effort was undertaken until mid-
81 August. Pigment analyses (Coupel et al. 2015) and light microscopy techniques ([http://malina.obs-
83 vlfr.fr/data.html](http://malina.obs-
82 vlfr.fr/data.html)) confirmed previous findings on phytoplankton community composition and
84 revealed that Prymnesiophyceae, Mamiellophyceae and Dinophyceae dominated offshore waters
85 while diatoms accounted for most abundance and biomass on the Mackenzie Shelf (Coupel et al.
86 2015). Within diatoms the cold-water ecotype of *Chaetoceros socialis* described recently as
87 *Chaetoceros gelidus* (Degerlund et al. 2012, Chamnansinp et al. 2013), several other *Chaetoceros*
88 taxa, and with lower abundances, *Thalassiosira nordenskiöldii*, and *Pseudo-nitzschia* spp.
89 prevailed (<http://malina.obs-vlfr.fr/data.html>). Molecular techniques [cloning/sequencing and
90 terminal-RFLP (T-RFLP) on the 18S rRNA gene] on photosynthetic populations (Balzano et al.
91 2012b) partially agree with pigment analyses and phytoplankton microscopy counts indicating that
92 Arctic *Micromonas* (Lovejoy et al. 2007) was the only photosynthetic picoplankter (< 2 µm)
93 detected in most stations, whereas nanoplankton (2-20 µm) genetic libraries were dominated by the
94 diatoms *C. gelidus* (referred therein as *C. socialis*) and *Chaetoceros neogracilis* in DCM and
95 surface waters, respectively (Balzano et al. 2012b).

95 Seasonal succession and geographic distribution of phytoplankton species have thus been
96 partially elucidated for the Beaufort Sea, but species level diversity has still not been fully assessed
97 for diatoms, due to the limited resolution power of the morphological and molecular methods
98 employed. Light microscopy, that has been applied in most the studies, does not allow the
99 observation of the fine ultrastructural details often required to distinguish diatom species. Similarly,
100 the 18S rRNA gene did not allow discrimination among some species of the genera *Chaetoceros*
101 and *Pseudo-nitzschia* H. Peragallo, which were well represented in the area (Balzano et al. 2012b).
102 Other ribosomal genes have a higher resolution power: the 28S rRNA gene can successfully
103 discriminate most of the species within the genera *Chaetoceros* (Kooistra et al. 2010) and *Pseudo-*
104 *nitzschia* (Lundholm et al. 2002) and is considered a good discriminatory molecular marker among

105 centric diatom species (Lee et al. 2013). A gene fragment extending from the 5' end of the 5.8S to
106 the 3' end of the helix III of ITS-2 (5.8S + ITS-2) has been proved to separate the 99.5 % of diatom
107 species (Moniz & Kaczmarek 2010).

108 Coupling culture isolation with morphological and genetic characterization allows detailed
109 species identification. This approach has been applied to photosynthetic flagellates collected during
110 the MALINA cruise. Photosynthetic pico- and nanoeukaryotic populations were dominated by
111 cultured microorganisms (Balzano et al. 2012b) and 104 strains belonging to the Chlorophyta,
112 Dinophyta, Haptophyta, Cryptophyta and Heterokontophyta divisions were isolated and
113 characterized by both light microscopy (LM) and 18S rRNA gene sequencing (Balzano et al.
114 2012a).

115 A recent study investigated Arctic dinoflagellates coupling morphological and genetic
116 approaches (Gu et al. 2013), but similar information on diatoms is missing. In the present paper, we
117 focus on diatom strains isolated from the Beaufort Sea. We combined LM, TEM, and SEM with
118 18S and 28S rRNA gene sequencing to identify the isolated strains. We also sequenced the ITS
119 operon of the rRNA gene from a number of *C. neogracilis* strains sharing highly similar 18S and
120 28S rRNA gene sequences to further investigate the occurrence of distinct genetic entities and we
121 reconstructed the secondary structure of the ITS-2 of these strains in order to predict their
122 reproductive isolation.

123

124

125 MATERIALS AND METHODS

126

127 *Phytoplankton sampling, isolation and maintenance.* Strains were isolated from seawater
128 samples collected during the MALINA (<http://www.obs-vlfr.fr/Malina>) cruise which sailed the
129 06/07/09 from Victoria (British Columbia, Canada) to the Beaufort Sea where an extensive
130 sampling effort was carried out in late summer from 1/08/09 to 24/08/09 (Table S1 in the
131 Supporting Information). Samples were collected with a bucket from surface waters in the North
132 Pacific and at different depths with Niskin bottles mounted on a CTD frame in the Beaufort Sea.
133 Phytoplankton strains were isolated both onboard and back in the laboratory (Table 1) as described
134 previously (Le Gall et al. 2008, Balzano et al. 2012a). Overall we isolated 75 diatom strains, 60 of
135 which are currently (March 2016) available from the Roscoff Culture Collection (RCC:
136 <http://www.roscoff-culture-collection.org/>). Most of the strains were isolated from the Beaufort Sea
137 but we also included four strains from the North Pacific sampled during the first leg of the
138 MALINA cruise for comparison purposes. The strains were maintained in K or K/2-medium
139 (Keller et al. 2009) with addition of silicate, prepared from sterile seawater at a salinity of 35 and
140 kept at 4°C at an irradiance of 50 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in a 12:12 light dark regime. Some of the
141 *C. neogracilis* strains were incubated at low light intensity (about 10 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) in f/2
142 medium (Guillard 1975) with nitrate supplied at a concentration 10-fold lower (88 μM) to induce
143 resting spore formation, since spore morphology can help species identification in the genus
144 *Chaetoceros* (Hasle & Syvertsen 1997).

145 *DNA extraction and PCR.* Genomic DNA was extracted from 75 MALINA strains as described
146 previously (Balzano et al. 2012a) using the NucleoSpin Tissue kit (Mackerey Nagel, Hoerd, t,
147 France) and following the instructions provided by the manufacturer.

148 The 18S rRNA gene, the Internal Transcribed Spacer (ITS) of the rRNA operon and the 28S
149 rRNA gene were then amplified by PCR on genomic DNA. For the 18S rRNA gene the primers 63f

150 (5'-ACGCTT-GTC-TCA-AAG-ATTA-3') and 1818r (5'-ACG-GAAACC-TTG-TTA-CGA-3')
151 were used (Lepère et al. 2011) as described previously (Balzano et al. 2012a).

152 The ITS region of the rRNA operon was amplified from 35 MALINA strains of *C. neogracilis*
153 (Table 1) and 3 Antarctic strains of *Chaetoceros* purchased from the National Centre for Marine
154 Algae and Microbiota (Bigelow, USA) and previously thought to belong to *C. neogracilis*
155 (CCMP187, CCMP189, and CCMP190; Table S2 in the Supporting Information). The ITS was
156 amplified using primers 329f (5'-GTG-AAC-CTG-CRG-AAG-GAT-CA-3') and D1R-R (5'-TAT-
157 GCT-TAA-ATT-CAG-CGG-GT-3') which correspond to the reverse complements of the reverse
158 primer for 18S 329r (Guillou et al. 2004) and the 28S forward primer D1R (Lenaers et al. 1989),
159 respectively. PCR condition included an initial incubation step at 95°C during 5 min, 35
160 amplification cycles (95°C for 1 min, 55°C for 45 s, and 72°C for 1 min 15 s) and a final elongation
161 step at 72 °C for 7 min. From 72 diatom strains, the 28S rRNA gene was amplified using primers
162 D1R (5'-ACC-CGC-TGA-ATT-TAA-GCA-TA-3') and D3Ca (5'-ACG-AAC-GAT-TTG-CAC-
163 GTC-AG-3') targeting the D1–D3 region of the nuclear LSU rRNA (Lenaers et al. 1989, Orsini et
164 al. 2002). PCR reactions were as follows: 30 amplification cycles of 94°C for 1 min, 55°C for 1
165 min 30 s, and 72°C for 1 min.

166 18S rRNA, ITS, and 28S rRNA amplicons were purified using Exosap (USB products, Santa
167 Clara, USA) and partial sequences were determined by using Big Dye Terminator V3.1 (Applied
168 Biosystems, Foster city, USA). The hypervariable V4 region (Dunthorn et al. 2012) of the 18S
169 rRNA gene was sequenced from all the strains using the internal primer Euk528f (Zhu et al. 2005),
170 whereas the primers 63f and 1818R were used to sequence the full 18S rRNA gene from selected
171 strains. The ITS region was sequenced using both forward and reverse primers described above
172 whereas the forward primer D1R was used to sequence the 28S rRNA gene. Sequencing was carried
173 out on an ABI prism 3100 sequencer (Applied Biosystems).

174 *Phylogenetic analysis.* V4 sequences were compared to those available in Genbank using
175 BLAST (blast.ncbi.nlm.nih.gov/Blast.cgi), aligned using ClustalW2

176 (<http://www.ebi.ac.uk/Tools/msa/clustalw2>) and then grouped into 17 different 18S genotypes
177 based on 99.5% sequences similarity, using the Bioedit software (Hall 1999). The full 18S rRNA
178 gene was sequenced from at least one strain per genotype (19 strains in total). For all the
179 phylogenetic trees shown in this paper, relationships were analyzed using Maximum Likelihood
180 (ML) and Neighbour Joining (NJ) methods (Nei & Kumar 2000) and bootstrap values were
181 estimated using 1,000 replicates (Felsenstein 1985) for both methods. MEGA5 software (Tamura et
182 al. 2011) was used to construct the phylogenetic trees based on the ML topology.

183 Full 18S rRNA sequences were aligned with reference sequences from Genbank
184 (<http://www.ncbi.nlm.nih.gov/nucleotide>, Table S2) for a total of 84 sequences using clustalW2 as
185 described above. Highly variable regions of the alignment were removed and the final dataset
186 contained 1,465 nucleotide positions. A Tamura Nei model (Tamura & Nei 1993) was selected as
187 the best model to infer both NJ and ML 18S phylogeny.

188 For the D1-D3 region of the 28S rRNA gene 64 sequences were aligned using clustalw2 and a
189 subset, containing at least one sequence per genotype, was used to construct three phylogenetic
190 trees (centric diatoms, pennate diatoms and *C. neogracilis* strains). Highly variable regions were
191 removed from the alignments. For the centric diatoms, the alignment included, 65 sequences and
192 504 positions and the phylogeny was inferred using a Kimura-2 model (Kimura 1980).

193 Phylogenetic relationships were then inferred as described above and 5 sequences from the genus
194 *Attheya* West were used as an outgroup and were then removed from the tree for clarity. For the
195 pennate diatoms, the alignment included 35 sequences and 490 nucleotide positions and the
196 phylogeny was inferred using a Tamura-Nei model (Tamura & Nei 1993) and sequences from the
197 genus *Attheya* were also used as an outgroup. A third phylogenetic tree was constructed for *C.*
198 *neogracilis*, which included 36 MALINA strains from this species, 1 sequence of the strain CPH9
199 identified as *Chaetoceros fallax* Proschkina Lavrenko, 3 GenBank sequences from the Antarctic
200 strains CCMP163, CCMP189 and CCMP190 (Table S2) and one sequence from *C. gelidus*

201 (RCC2271) which was used as an outgroup. The analysis was performed on 41 sequences for a total
202 of 590 positions using a Kimura-2-parameter model.

203 We also sequenced the ITS operon of the rRNA gene from the MALINA strains affiliated to *C.*
204 *neogracilis* as well as the Antarctic strains attributed by CCMP to *C. neogracilis*. Since the 5.8S is a
205 region highly conserved at interspecific level, we identified the boundary between ITS-1 and 5.8S
206 based on 5.8S sequences from other *Chaetoceros* species (Moniz & Kaczmarska 2010) available in
207 GenBank. We then constructed a phylogenetic tree based on the ITS-1 and another phylogenetic
208 tree consisting in a region starting at the 5' end of 5.8S and ending in the conserved motif of helix
209 III of ITS-2. Some sequences did not cover the entire ITS length and were excluded from the
210 alignment of either the ITS-1 or the 5.8S/ITS-2. The ITS-1 alignment included 30 sequences and
211 227 nucleotide positions and was analysed using a Jukes Cantor model (Jukes & Cantor 1969). For
212 the 5.8S/ITS-2 alignment the end of helix III was annotated based on the secondary structure of the
213 ITS-2 from *T. weissflogii* (Grunow) Fryxell & Hasle (Sorhannus et al. 2010), which is the species
214 most closely related to the genus *Chaetoceros* for which the secondary structure of the ITS-2 has
215 been reconstructed. The final alignment included 30 sequences and 384 nucleotide positions and
216 both ML and NJ phylogenies were inferred using a Kimura-2 model (Kimura 1980). The ITS could
217 not be sequenced from the strain MALINA E43.N2, but it was attributed to Clade II based on its
218 28S sequence. Similarly since both the ITS-1 and the 5.8 + ITS-2 sequences from RCC2268,
219 RCC2277 and RCC2318 were not sufficiently long to be included in the ITS-1 and the 5.8S/ITS-2
220 alignments, a neighbour joining phylogenetic tree for the entire ITS fragment which included 34
221 sequences for a total 483 positions (Fig. S1 in the Supporting Information) was constructed in order
222 to identify the genetic clade of these strains.

223 *ITS-2 structure prediction.* To characterize our MALINA strains of *C. neogracilis* in deeper
224 detail we reconstructed the secondary structure of the ITS-2 operon of the rRNA. The ITS-2
225 boundaries were then annotated using Hidden Markov Models of the flanking 5.8S and 28S regions
226 (Keller et al. 2009). The secondary structure of the ITS-2 was first inferred for the strain RCC2014

227 using the RNA structure program (Mathews et al. 2004) and then transferred onto other
228 *Chaetoceros* sequences through homology modelling (Wolf et al. 2005) using the ITS-2 database
229 (Merget et al. 2012).

230 *Microscopy.* At least one strain per genotype, for a total of 61 strains (Table 1), was observed
231 and photographed in light microscopy. Cells were harvested during the exponential phase of their
232 growth and observed using an Olympus BX51 microscope (Olympus, Hamburg, Germany) with a
233 100X objective using differential interference contrast (DIC). Cells were imaged with a SPOT RT-
234 slider digital camera (Diagnostics Instruments, Sterling Heights, MI, USA). Micrographs are
235 available at <http://www.roscoff-culture-collection.org> for a large set of strains.

236 Selected strains, covering most genetic diversity based on both 18S and 28S rRNA, were
237 observed using Light Microscopy (36 strains), TEM (25 strains) and/or SEM (28 strains) at
238 Stazione Zoologica Anton Dohrn (Table 1). To remove organic matter, samples were treated with
239 nitric and sulfuric acids (1:1:4, sample:HNO₃:H₂SO₄), boiled for a few seconds and washed with
240 distilled water. LM observations were performed using a Zeiss Axiophot 200 equipped with a
241 Axiocam Digital Camera (Carl Zeiss, Oberkochen, Germany). Acid-cleaned material was mounted
242 on Formvar- coated grids and observed with a LEO 912AB transmission electron microscope (LEO,
243 Oberkochen, Germany) and/or mounted on stubs, sputter-coated with gold-palladium and observed
244 with a JEOL JSM-6500F SEM (JEOL-USA Inc., Peabody, MA, USA). Fixed samples not subjected
245 to cleaning were placed on Nuclepore 3 µm pore size (Nuclepore, Pleasanton, CA, USA)
246 polycarbonate filters, rinsed with distilled water, dehydrated in an ethanol series (25, 50, 75, 95, and
247 100%), and critical-point-dried. Dried filters were mounted on stubs, sputter-coated and observed
248 with SEM.

249 RESULTS

250

251 In the present study, we characterized 75 diatom strains using a combination of morphological
252 and molecular techniques (Table 1). We sequenced the V4 region of the 18S rRNA from all the
253 strains and then we sequenced the full 18S rRNA from at least one strain from each unique
254 genotype. Moreover we sequenced the 28S rRNA from most of our strains and the ITS operon of
255 the rRNA from all the strains affiliated to *C. neogracilis*. The strains grouped into 17 genotypes
256 based on 18S and 28S rRNA phylogenies (Figs. 1 and 2). 28S rRNA and ITS analyses indicate that
257 36 strains of *C. neogracilis* sharing identical 18S rRNA gene sequence make up 4 distinct genetic
258 clades (Figs. 2 and 3). The most represented genera were *Chaetoceros* and *Thalassiosira*.

259 *Bacillariaceae*. We isolated 9 Bacillariaceae strains from the genera *Cylindrotheca*, *Nitzschia*
260 and *Pseudo-nitzschia*. The 18S rRNA gene (Fig. 1) discriminated the different *Cylindrotheca* and
261 *Nitzschia* representatives but was poorly resolutive for the different *Pseudo-nitzschia* species.

262 *Cylindrotheca closterium* (Ehrenberg) Lewin & Reimann.

263 Cells are 85 to 108 µm long, fusiform with rostrated ends and possess two chloroplasts (Hasle
264 1964, Jahn & Kusber 2005). The valve face is unperforated, transversed by transapical slightly
265 silicified ribs. The central raphe is interrupted by a central nodule. The fibulae (13-17 in 10 µm) are
266 narrow, irregularly spaced, and joined directly to the valve face (Fig. 4A; Hasle 1964, Jahn &
267 Kusber 2005).

268 *Cylindrotheca closterium* was considered as a cosmopolitan species but it was demonstrated to
269 constitute a species complex of similar morphotypes belonging to different genetic lineages (Haitao
270 et al. 2007). It has been repeatedly observed in the Arctic (Table 2). The 18S rRNA gene sequence
271 from *C. closterium* strain RCC1985 (Fig. 1) groups with the other *C. closterium* sequences forming
272 a moderately-supported clade (sequence similarity > 97.8 %), but does not cluster to any of the two
273 lineages described to date for the *C. closterium* species complex (Haitao et al. 2007). The 28S

274 rRNA gene sequence from *C. closterium* strain RCC1985 (Fig. 2A) branches with two other
275 sequences from *C. closterium*.

276 *Nitzschia pellucida* Grunow.

277 Cells (apical axis: 35 μm ; transapical axis: 3.0-3.5 μm) are solitary and possess two chloroplasts.
278 Cells are lanceolate, tapering towards the poles, in valve view (Fig. 4B), and rectangular when
279 observed in girdle view. The densities of fibulae and striae are 12-15 and 35-40 in 10 μm ,
280 respectively. Each stria contains one row of rounded poroids. A central larger interspace is present
281 (Fig. 4, C and D).

282 *Nitzschia pellucida* has been previously reported in Arctic and Antarctic waters but also in
283 European freshwater environments (Table 2). The 18S rRNA gene sequence from *N. pellucida*
284 strain RCC2276 is highly related to that of *Nitzschia dubiiformis* (99.6 % sequence identity) and
285 branches with other *Nitzschia* species (Fig. 1). The 28S rRNA gene sequence from *N. pellucida*
286 strain RCC2276 groups with *Nitzschia laevis* and *N. pellucida* from GenBank (sequence identity
287 97.5 and 97.4, respectively). This clade branches with different *Nitzschia* and *Cylindrotheca* species
288 (Fig. 2A), which supports the assertion of Lundholm et al. (2002) describing the genus *Nitzschia* as
289 polyphyletic.

290 *Pseudo-nitzschia granii* (Hasle) Hasle.

291 Cells (apical axis: 17-25 μm ; transapical axis: 1.4-1.8 μm) have two chloroplasts and colonies
292 were not observed in culture conditions. Valves are lanceolate with a central swelling, one side of
293 the valves is linear and the other convex (Fig. 4E). Apices are rounded. The striae (54-55 in 10 μm)
294 are composed of a single row of poroids divided in 5-7 sectors. In the strain RCC2006, most of the
295 valves have striae barely silicified that lack complete poroids (Fig. 4F) or have few poroids entirely
296 formed (Fig. 4G). The fibulae (16-18 in 10 μm) are irregularly spaced and the central interspace is
297 absent.

298 *Pseudo-nitzschia granii* has been reported in northern cold waters, including Arctic and subarctic
299 regions (Table 2).

300 *Pseudo-nitzschia arctica* Percopo & Sarno.

301 Four *Pseudo-nitzschia* strains isolated during the MALINA cruise have been recently described
302 as a new species, *Pseudo-nitzschia arctica* (Percopo et al. 2016). Cells occur in colonies and each
303 cell overlaps the next sibling cell for ca 1/8 of its length (Fig. 4H). Cells (apical axis: 26-60 μm ;
304 transapical axis: 1.6-2.5 μm) are lanceolate in valve view. The valve ends are broadly pointed. The
305 fibulae are not always regularly spaced. The two central fibulae have a larger interspace and the
306 raphe is here interrupted by a central nodule (Fig. 4I). The densities of fibulae and interstriae are 17-
307 24 and 34-39 in 10 μm , respectively. The striae contain 1 row of rounded poroids, 5-6 poroids in 1
308 μm . Each poroid most often contains 1-6 sectors. Some striae are simply composed of more lightly
309 silicified areas without any perforations.

310 *Pseudo-nitzschia arctica* seems to have a distribution confined to the northern polar area,
311 possibly representing one of the endemic components of the Arctic diatom flora (Percopo et al.
312 2016).

313 *Pseudo-nitzschia arctica* and *P. granii* share highly similar 18S rRNA gene sequences (99.6 %
314 sequence identity, Fig. 1) and the two species can be better separated using 28S rRNA phylogeny
315 (Fig. 2A) where their sequences differ by 1.2 % sequence identity.

316 *Fragilariaceae.*

317 *Synedropsis hyperborea* (Grunow) Hasle, Medlin & Syvertsen.

318 Cells (apical axis: about 55 μm ; transapical axis: 2.7-3.5 μm) are lanceolate in valve view (Fig.
319 4J). No colonies were observed. The uniseriate striae (22-23 in 10 μm) are parallel towards the
320 apices and alternate in the some parts of the valve (Fig. 4K). The apical fields are composed of 5-7
321 slits (Fig. 4, L-N) slightly different from that reported in the original description (4-6 slits, Hasle et
322 al. 1994). A single rimoportula is located two or three striae from one of the two valve apices (Fig.
323 4L). The rimoportula opens externally into a hole larger than the surrounding areolae (Fig. 4, M and
324 N).

325 *Synedropsis hyperborea* is typical of the Northern cold region and it is commonly reported in
326 Arctic waters (Table 2).

327 Fragilariaceae taxonomy was not well resolved based on 18S rRNA gene since the sequence
328 from *S. hyperborea* strain RCC2043 shares very high similarities with a sequence from Genbank
329 affiliated to *S. hyperborea* (99.9 %) as well as *Synedra minuscula* (99.9 %), *Fragilaria* sp. (99.9 %)
330 and *Grammonema striatula* (99.5 %, Fig. 1). MALINA strains RCC2043 and RCC2520 belonging
331 to *S. hyperborea* share identical 28S rRNA gene sequences and group with a sequence from
332 *Synedropsis hyperboreoides* from GenBank (98.5 % sequence identity). The 28S rRNA gene
333 sequences from these strains are also related to *Thalassionema frauenfeldii* and 3 *Fragilaria* species
334 (Fig. 2A).

335 *Attheyaceae*. *Attheya septentrionalis* (Østrup) Crawford.

336 Cells (apical axis: 3.5-6.4 µm; perivalvar axis: 7-11.7) are solitary and bear four slightly wavy
337 horn-like projections (Fig. 5, A and B). One or two plate-like chloroplasts are present. Valves are
338 almost circular and lack the rimoportula (Fig. 5C). The length of the horns is variable (12-35 µm)
339 and the ratio between horn length and cell diameter ranges from 2.9 to 4.4. The number of
340 longitudinal strips can be 3 or 4 in both examined strains (Fig. 5, D and E).

341 *Attheya septentrionalis* is distributed in the northern cold region and it is common in Arctic
342 waters (Table 2). The 18S rRNA gene from the MALINA strains RCC1986 and RCC2042 branches
343 with that of sequences of *A. septentrionalis* (99.9 % sequence identity) and *Attheya longicornis*
344 (99.8 %) from GenBank and is related to sequences from three *Biddulphia* spp. (Fig. 1). The 28S
345 rRNA gene from the two MALINA strains is also highly related to that of sequences of *A.*
346 *septentrionalis* and *A. longicornis* (≈ 98 % sequence identity, Fig. 2A).

347 *Thalassiosiraceae*. We isolated 12 Thalassiosiraceae strains (Table 1) affiliated to the genera
348 *Thalassiosira*, *Porosira*, and *Shionodiscus*. Both 18S and 28S rRNA gene allowed the
349 discrimination of the different species found here (Figs. 1 and 2B).

350 *Thalassiosira gravida* Cleve.

351 Cylindrical cells (diameter: 28.5-30.5 μm) held in colonies by a single thick thread composed of
352 several strands (Fig. 5F). A number of fuloportulae (or strutted processes, 11-15) are grouped in a
353 central cluster and several fuloportulae are scattered on the valve face. The marginal fuloportulae
354 are arranged to form 3-4 rings placed between the margin of the valve face and the mantle. A single
355 rimoportula (or labiate process) is located within the inner ring of marginal fuloportulae (Fig. 5G).
356 Different valves have a variable degree of silicification, but in general the areolae are well-formed
357 on the margins of the valve (16-20 in 10 μm) and poorly developed in the central part, where
358 siliceous radial ribs separate perforated areas.

359 *Thalassiosira gravida* is regarded as a bipolar, cold to temperate water species and it has been
360 previously observed in Arctic and Antarctic waters (Table 2).

361 The 18S rRNA gene sequence from *T. gravida* strain RCC1984 clusters with sequences from
362 both *T. gravida* and *T. rotula* (sequence identity > 99.5 %) and is highly related with a sequence
363 from *Thalassiosira eccentrica* (99.3 % sequence identity, Fig. 1). The 28S rRNA gene sequences
364 from both our strains of *T. gravida* group with two other sequences from *T. gravida* and are highly
365 related to 2 sequences from *T. rotula* (99.2 % sequence identity, Fig. 2B).

366 *Thalassiosira cf. hispida* Syvertsen.

367 Cells (diameter: 6.5-13.5 μm) possess several chloroplasts, and form colonies of few cells (3-4
368 cells) connected by one central thread. The areolae (30 in 10 μm) have a similar size on both valve
369 face and mantle. One ring of marginal fuloportulae (5 in 10 μm) and one central fuloportula are
370 present on the valve face (Fig. 5H). The marginal fuloportulae have long external tubes (Fig. 5, H-
371 K). All the fuloportulae have four satellite pores at their base (Fig. 5I). The rimoportula is
372 positioned slightly inside the ring of marginal fuloportulae, between two of them. It can be either
373 closer to one of them or in the middle. A broad hyaline margin is present. Short and minute spines
374 and hairs emerge throughout the valve (Fig. 5I). The girdle is formed by a valvocopula, a copula
375 and several open bands. The valvocopula has a broad abvalvar imperforated rim and one advalvar
376 row of areolae (Fig. 5J). MALINA strain of *T. cf. hispida* is morphologically very similar to the

377 original description of *T. hispida* but possesses a higher number of areolae (18 and 24-26 in 10 µm
378 on valve face and mantle, respectively, in Syvertsen 1986). Very similar is the dense covering of
379 spinules on the valve surface, which however is not specific for *T. hispida*, but can be developed to
380 a lesser extent in other *Thalassiosira* species, and the presence of a broad hyaline margin on the
381 valve and a valvocopula with a wide non-pierced edge.

382 *Thalassiosira hispida* has only been reported in northern cold water regions (Table 2). 18S rRNA
383 gene sequences from *T. hispida* are not available on the GenBank and the 18S rRNA gene sequence
384 from our strain RCC2521 clusters with sequences of *Thalassiosira allenii* (98.5 % sequence
385 identity) and *Thalassiosira angulata* (98.6 %, Fig. 1). The 28S rRNA gene sequence from
386 RCC2521 (Fig. 2B) groups with *T. allenii* (97.5 %), *Thalassiosira aestivalis* (97.1 %) and *T.*
387 *nordenskiöldii* (96.5 %) but the clade is poorly (< 50 % ML and NJ) supported.

388 *Thalassiosira minima* Gaarder.

389 Cells (diameter: 4.5-13µm) have two chloroplasts and do not form colonies under our culture
390 conditions. In girdle view, cells are rectangular with a perivalvar axis generally shorter than the cell
391 diameter and with a valve face slightly depressed in the centre (Fig. 5L). The areolae (30-35 in 10
392 µm) are hexagonal in shape (Fig. 5M). On the valve, a ring of marginal fultoportulae (4-6 in 10 µm)
393 with short external tubes and one or two central fultoportulae are present (Fig. 5, M and N). Five
394 fultoportulae have been occasionally observed in one single valve (Fig. 5O). A large rimoportula is
395 placed between two marginal fultoportulae, slightly closer to one of them (Fig. 5, M and N). Each
396 marginal fultoportula is accompanied with a small external labiate-shaped protrusion (Fig. 5P). The
397 species has a worldwide distribution (Table 2) and it is reported for the first time in the Arctic
398 Ocean.

399 The 18S rRNA gene sequence from our *T. minima* strain RCC2265 is highly similar to that of
400 the *T. minima* sequence from the strain CCMP990 (99.7 %, Fig. 1). Our strains of *T. minima* from
401 both the Beaufort Sea and the North Pacific Ocean (Table 1) share highly similar 28S rRNA gene
402 sequences with the Antarctic strain RCC2707 (99.1 %) and group with the *T. minima* strain

403 CCMP990 forming a well-supported clade (Fig. 2B). Consistent with the 18S rRNA gene
404 phylogeny, *Thalassiosira curviseriata* is the species most closely related to all the *T. minima*
405 strains.

406 *Thalassiosira nordenskiöldii* Cleve.

407 Cells (diameter: 12-15 µm) possess several chloroplasts and form long colonies connected by a
408 central thread (Fig. 6A). Areolae are 17-18 on valve face and 18-20 in 10 µm on mantle (Fig. 6B).
409 Valves are characterized by a pronounced concavity in the centre, a high (4-6 areolae) and oblique
410 mantle, a marginal ring of fultoportulae (3-4 in 10 µm) with long external tubes bearing a terminal
411 collar, one central fultoportula and one rimoportula positioned within two marginal fultoportulae
412 (Fig. 6, B and C).

413 *Thalassiosira nordenskiöldii* is a species typical of northern cold to temperate regions, common
414 in Arctic waters (Table 2).

415 The 18S rRNA gene sequence from strain RCC2000 groups with sequences from *T. aestivalis*
416 and *T. nordenskiöldii* forming a well-supported clade (Fig. 1). *Thalassiosira nordenskiöldii*
417 RCC2000 shares identical 28S rRNA gene sequence with another *T. nordenskiöldii* strain from the
418 GenBank and highly similar 28S sequence (99.8 %) with *T. nordenskiöldii* RCC2021. These
419 strains form a clade with a sequence from *T. aestivalis* (Fig. 2B).

420 *Porosira glacialis* (Grunow) Jørgensen.

421 Cells (diameter: 30-40 µm) are cylindrical, possess several chloroplasts and can form short
422 colonies (2-3 cells; Fig. 6, D and E). Numerous fultoportulae are scattered over the valve surface (3-
423 4 in 10 µm). The striae (24-27 areolae in 10 µm) are wavy and radially arranged. A central annulus
424 is present and a large rimoportula process is situated inside the margin of the valve (Fig. 6F).

425 *Porosira glacialis* is reported in Arctic and Antarctic waters (Table 2).

426 RCC2039 18S rRNA is identical with that from the Antarctic strain CCMP1099 (Fig. 1). The
427 28S rRNA gene sequence from the MALINA strain RCC2039 is highly related, but not identical
428 (99.6 %), to that of the two Antarctic strains CCMP1099 and RCC2709 (Fig. 2B).

429 *Shionodiscus bioculatus* (Grunow) Alverson, Kang & Theriot.

430 Cells (diameter: 23-41 μm) are solitary and possess a large number of discoid chloroplasts (Fig.
431 6G). The perivalvar axis is generally longer than the diameter. The valve face is slightly convex and
432 the mantle is rounded. The areolation is fasciculate (20-23 areolae in 10 μm) with a single
433 fulcrotortula in the valve centre and a subcentral rimotortula (Figs. 6, H and I). The marginal
434 fulcrotortulae (4-7 μm apart) have internal tube-like projections and no external extensions. Strain
435 RCC1991 is the first representative of *S. bioculatus* sequenced to date, both 18S and 28S rRNA
436 gene sequences from this strain group with sequences of *Shionodiscus oestrupii* and *Shionodiscus*
437 *ritscheri* (Figs. 1 and 2B).

438 *Cymatosiraceae*.

439 *Arcocellulus cornucervis*. Hasle, von Stosch & Syvertsen.

440 Cells are solitary, very small (apical axis: 3.0–3.5 μm ; perivalvar axis: 1.4-1.7 μm ; transapical
441 axis: 1.7-2.2 μm) and slightly curved in broad girdle view. Each cell possesses two different valves,
442 a process valve and a pili valve, which are convex and concave, respectively, in larger cells (Fig. 6,
443 J and K). Each valve has two ocelluli (Fig. 6, K and L). The pili cross each other and bear
444 conspicuous branches (Fig. 6J). The process valve possesses a central process (Figs. 6K and 7A). A
445 marginal row of poroids is always present along the margin of the valve and a variable number of
446 poroids can be present on the valve face. The basal siliceous layer may be smooth or ornamented by
447 costae which can be indistinct or more convoluted (Fig. 6K). Costae seem to be more pronounced in
448 process valves. Patches of short spinules can be present near the pilus base (Fig. 7A).

449 *Arcocellulus cornucervis* has been reported in temperate and cold waters of both hemispheres,
450 including Arctic Ocean (Table 2).

451 18S phylogeny could not discriminate *Arcocellulus* spp. from the closely related genus
452 *Minutocellulus* (Fig. 1). The 18S rRNA gene sequence from *A. cornucervis* RCC2270 is indeed
453 highly related to two sequences from *Minutocellus polymorphus* (99.5 % sequence identity) and
454 both form a well-supported (96% ML, 100 % NJ) clade which branches with that of other

455 representatives from the family Cymatosiraceae, namely *Papiliocellulus elegans*, *Cymatosira*
456 *belgica*, and *Brockmanniella brockmanni* (Fig. 1).

457 The 28S rRNA gene of *A. cornucervis* strain RCC2270 is closely related to that of two
458 unidentified Cymatosiraceae (95 and 95.9 % sequence identity) isolated from temperate waters
459 (Fig. 2B).

460 *Hemiaulaceae*.

461 *Eucampia groenlandica* Cleve.

462 Cells (apical axis: 7-24 μm) are rectangular in girdle view, slightly silicified and possess several
463 chloroplasts. Cells form colonies which can be straight or slightly curved in broad girdle view with
464 square to hexagonal apertures (Fig. 7B). A rimoportula is present on the centre of the valve (Fig.
465 7C).

466 *Eucampia groenlandica* was first reported from Baffin Bay in Davis Strait and is considered
467 typical of the northern cold waters (Table 2).

468 The 18S rRNA gene sequence from *E. groenlandica* strain RCC1996 groups with sequences of
469 *Eucampia zodiacus* (99.2 %) and *Eucampia antarctica* (99.0 %) forming a well-supported clade
470 (Fig. 1). The 28S rRNA gene from our strains is related to a sequence from *E. zodiacus* (96.9 %
471 sequence identity, Fig. 2B).

472 *Chaetocerotaceae*. We isolated 45 strains of the genus *Chaetoceros* and using the 28S rRNA
473 (Fig. 2B, and 3A) and ITS phylogeny (Fig. 3, B and C) we grouped these strains into 6 genotypes, 2
474 of them corresponding to the species *Chaetoceros decipiens* and *C. gelidus* respectively, and 4 other
475 being closely related genotypes affiliated to *C. neogracilis*.

476 *Chaetoceros decipiens* Cleve.

477 Cells (apical axis: 11-22 μm) were generally solitary in culture conditions but a few colonies
478 have been observed (Fig. 7, D and E). Each cell possesses several chloroplasts.

479 Chains are straight and the apertures are elliptical. All setae lie in the apical plane. The intercalary
480 setae emerge from the valve margin without a basal part and may fuse for a shorter or longer

481 distance. Terminal setae are U or V shaped (Fig. 7, D and E). The valve, with a high mantle, is
482 almost flat in girdle view (Fig. 7, F and G). Valves have a central annulus from which irregular ribs
483 radiate and are perforated with small poroids. The mantle is high and a marginal ridge is present
484 between the valve face and mantle (Fig. 7F). Terminal valves possess a very small central process
485 with a short external projection (Fig. 7H). Girdle bands are ornamented with parallel transverse
486 costae interspaced by hyaline areas with scattered small poroids (Fig. 7I). The setae are polygonal,
487 mostly four-sided, in cross section, with spines on the edges and a single longitudinal row of large
488 pores on each side.

489 *Chaetoceros decipiens* is a cosmopolitan species, common in arctic waters (Table 2).

490 The 18S rRNA gene sequence from our strain of *C. decipiens* (RCC1997) groups with a
491 GenBank sequence from *Chaetoceros cf. lorenzianus* (97.1 % sequence similarity, Fig. 1) and,
492 similarly, the 28S rRNA gene is closely related to GenBank sequences from *Chaetoceros*
493 *lorenzianus* (99.2 %), and groups with *Chaetoceros affinis* and *Chaetoceros diadema* (Fig. 2B).

494 *Chaetoceros gelidus* Chamnansinp, Li, Lundholm & Moestrup.

495 Cells (apical axis. 4-12 μm) with a single lobed chloroplast are joined in curved chains (Fig. 7J).
496 Several chains group together forming a spherical colony (Fig. 7 K). The setae emerge inside the
497 valve margin and merge after a short basal part forming narrow hexagonal apertures (Fig. 7L). In
498 valve view, the valve is circular to oval, in girdle view it is slightly concave with a small central
499 inflexion (Figs. 7L and 8A). Generally the cells have three short curved setae and one long straight
500 seta. The short setae have densely spirally arranged spines occurring throughout its length. In
501 contrast the long straight seta does not exhibit spines on its basal part, whereas on its distal part it
502 possesses spines which are more distant between each other (Fig. 8B). Both valves from each
503 resting spore are convex and smooth.(Fig. 8C). The crest reported in the original description
504 (Chamnansinp et al. 2013) is absent here. Variability in spore morphology of *C. gelidus* was
505 already reported (Degerlund et al. 2012, therein as *C. socialis*, northern strains).

506 The species has been reported from northern cold waters, including Arctic Ocean (Table 2).

507 The 18S sequence of *C. gelidus* clusters with a sequence of *C. socialis* (97.2 % sequence
508 identity, Fig. 1) and 28S rRNA sequences are identical to that of the type strain of *C. gelidus* (Fig.
509 2B).

510 *Chaetoceros neogracilis* (Schütt) VanLandingham.

511 Twenty-eight of the 36 strains of *C. neogracilis* isolated here have been observed by LM and
512 photographs are available for most of them (<http://www.roscoff-culture-collection.org>). Seven
513 strains have been further examined using EM (Table 1). Cells are generally solitary (Fig. 8, D-F)
514 but short colonies (3-6 cells) have been occasionally observed (Fig. 8G) in 9 strains. Cells are
515 relatively small (apical axis: 4-12 μm) and possess a single lobed chloroplast (Fig. 8, D-G). No
516 significant morphological and ultrastructural difference has been observed among the different
517 strains, with the exception of a certain variability in the orientation of the setae. As single cells,
518 some strains have straight setae diverging at an angle of 45° , whereas others have setae
519 perpendicular to pervalvar axis, and others have more curved setae (Fig. 8, D-F), but this variability
520 might be associated to the different cell sizes of the strains. In the colonies, cells are joined to form
521 straight chains and they are separated by apertures varying from elliptically shaped (Fig. 8, G and
522 H) to narrow slits (not shown). Terminal setae are U or V shaped. Valves are ornamented with
523 irregular costae originating from a central annulus. In the terminal valves, a slit-like process is
524 located in the centre of the annulus and it bears an external flattened tube (Fig. 8, I and J). The
525 central process is absent in the intercalary valves of the colonies, confirming that the chains are real
526 colonies and not cells in division (Fig. 8K). Intercalary setae originate from the valve apices, cross
527 immediately at the chain margin and diverge running in different directions (Fig. 8, H and L). The
528 setae are circular in cross section. They are composed by long spiral costae ornamented with
529 arrowhead-shaped spines (about 2 spines per $1\ \mu\text{m}$) and interconnected by short transverse costae
530 (Fig. 8, M and N). Spores were not observed in any of the tested strains.

531 The name *C. neogracilis* (basonym: *C. gracile* Schütt) has been attributed almost
532 indiscriminately to many small, unicellular *Chaetoceros* taxa collected worldwide (see Rines &

533 Hargraves 1988 for a discussion). The specific epithet can be found in the literature spelled as *C.*
534 *gracile* or *C. neogracile*, because the genus *Chaetoceros* was considered to be neutral, rather than
535 masculine. However the genus is currently recognised as a masculine word and the correct name of
536 the species is *C. neogracilis*. In more recent years, the species has been consistently reported as a
537 significant component of microbial communities in Arctic and Baltic (Table 2) as well as Antarctic
538 regions.

539 All the *C. neogracilis* strains isolated during the MALINA cruise share 100% identity in the V4
540 region of the 18S rRNA gene (data not shown). The full 18S rRNA gene has been sequenced for
541 strains RCC2016 and RCC2318. These two MALINA strains share identical 18S rRNA gene
542 sequence with the two Arctic strains ArM0004 e ArM0005 and form a well-supported clade with
543 the sequence from the Antarctic strain AnM0002 (98.9 % sequence identity, Fig. 1). The 28S rRNA
544 gene sequences from the MALINA strains of *C. neogracilis* cluster together (Fig. 2B) as well as
545 with a GenBank sequence from the Baltic strain CPH9 attributed to *C. fallax* (Chamnansinp et al.
546 2013) and have a sister clade which includes the sequences from three Antarctic strains (CCMP163,
547 CCMP189 and CCMP190). All these sequences branch with *Chaetoceros tenuissimus* forming a
548 well-supported clade (Fig. 3A).

549 *Genetic diversity of Chaetoceros neogracilis strains.* The MALINA strains of *C. neogracilis*
550 shared highly similar although not identical 28S rRNA gene sequence. Sequences can diverge by up
551 to 0.5 %. Both ITS markers as well as 28S rRNA gene indicate significant differences between the
552 Arctic and the Antarctic strains (Fig. 3), since the two groups form two separate branches. For
553 example the Arctic *C. neogracilis* RCC2014 shares with the Antarctic strain *Chaetoceros* sp.
554 CCMP189 95 %, 86 %, and 85 % sequence similarity for the 28S, ITS-1, and 5.8S + ITS-2,
555 respectively. The MALINA strains of *C. neogracilis* form four different clades based on all the
556 three markers used. Overall, based on either or both 28S rRNA (Fig. 3A) and ITS phylogeny (Figs.
557 3, B and C, S1), 20 strains belong to Clade I, 8 to Clade II, 2 to Clade III and 6 to Clade IV (Table
558 1). The 28S rRNA gene phylogeny (Fig. 3A) separates the *C. neogracilis* strains in two groups,

559 both with high (> 75 % in both ML and NJ) bootstrap support. One group consists of *C. neogracilis*
560 Clade I, whereas the second group includes the other 3 clades. Specifically strains from Clade II are
561 at the base of the group from which Clade III and Clade IV emerge with moderate (> 50 %) support
562 in both ML and NJ (Fig. 3A). The strain CPH9 falls within Clade II and the Antarctic strains
563 CCMP163, CCMP189 and CCMP190 are fully separated from *C. neogracilis*. Both ITS-1 and 5.8S
564 + ITS-2 trees includes 27 Arctic sequences from *C. neogracilis*, with 15 of them forming Clade I, 4
565 strains belonging to Clade II, 2 strains to Clade III, and 6 strains to Clade IV. Strains from each
566 clade cluster between them with moderate support in ITS-1 phylogeny and Clade II, Clade III, and
567 Clade IV group together with high bootstrap support (Fig. 3B). In 5.8S+ITS-2 phylogeny Clade II
568 and Clade III are highly supported, whereas Clade I and Clade IV are moderately supported; Clade
569 III groups with Clade I and some differences occur between the different strains from Clade II (Fig.
570 3C).

571 *Secondary structure of ITS-2.* We predicted the secondary structure of ITS-2 rRNA for our
572 strains of *C. neogracilis* to further investigate their genetic differences. We determined
573 Compensatory Base Changes (CBC) and Hemi-CBC in positions paired in the helices of the
574 secondary structure according to Coleman (2009). The secondary structure of ITS-2 from our
575 strains exhibits four helices (I, IIa, III and IV) typical of all eukaryotes (Coleman 2009) as well as
576 an additional helix (IIb) located between helix IIa and helix III (Fig. 9). Differences in the ITS-2
577 sequences from our strains occur at 14 positions, 9 of them located in paired positions of the
578 helices. This variability in paired positions consists in Hemi-CBC for 6 nucleotides, and CBC for 2
579 nucleotides. Two hemi-CBC occur in helix I (GC ↔ AC, and CG ↔ UG), 3 in helix III (CG ↔
580 UG, GC ↔ GU, GU ↔ AU), and 1 in helix IV (GU ↔ GC). Moreover 1 CBC occurs on helix IIa
581 between clade I and II (AU) versus clade IV (GC), with clade III showing a Hemi-CBC (GU)
582 towards the other three clades (Fig. 9).

583

584 DISCUSSION

585

586 *Combining microscopy and genetic data.* The combination of morphological and molecular
587 approaches on phytoplankton strains isolated during the MALINA cruise allowed the
588 characterization of cultured diatoms from the Beaufort Sea. To date about 10⁴ species have been
589 described based solely on their morphology (Guiry 2012) and the application of molecular
590 approaches during the last decade revealed a considerable genetic diversity within key planktonic
591 morphospecies such as *Asterionellopsis glacialis* (Castracane) Round (Kaczmarska et al. 2014),
592 *Leptocylindrus danicus* Cleve (Nanjappa et al. 2013), *Pseudo-nitzschia pseudodelicatissima*
593 (Lundholm et al. 2003, Lundholm et al. 2006, Amato & Montresor 2008, Lundholm et al. 2012,
594 Lim et al. 2013, Orive et al. 2013), and *Skeletonema costatum* (Sarno et al. 2005, Sarno et al. 2007,
595 Kooistra et al. 2008). It has been suggested that the number of extant diatom species exceeds by
596 one order of magnitude those described to date (Mann & Vanormelingen 2013).

597 Our work provides both 18S and 28S rRNA gene sequences validated with detailed
598 morphological and ultrastructural information for 17 morphotypes. Both genes have been sequenced
599 here for the first time for 6 diatom species (*A. cornucervis*, *C. decipiens*, *E. groenlandica*, *S.*
600 *bioculatus*, and *T. cf. hispida*). The 18S gene of *C. gelidus*, *N. pellucida*, and *P. arctica* has been
601 also sequenced for the first time. Moreover, most of the gene sequences obtained from the Arctic
602 strains were different from sequences from conspecific strains collected from different geographic
603 areas that are available in GenBank. Finally, we investigated the genetic rRNA diversity of 36
604 *Chaetoceros* strains sharing the same 18S gene sequence, and clarified the identity of *C.*
605 *neogracilis*, a taxon that dominated genetic libraries from the Beaufort Sea.

606 *Genetic markers and species delimitation.* The taxonomic resolution of the genetic markers used
607 here was different according to the genus investigated, but it also varied within a given genus,
608 depending on the phylogenetic distance existing between congeneric species.

609 The 18S rRNA gene can successfully discriminate species within the genus *Nitzschia* (Rimet et
610 al. 2011) and the *C. closterium* species complex (Haitao et al. 2007). Both 18S and 28S rRNA
611 genes are commonly used for the taxonomic identification of *Thalassiosira* species (Kaczmarska et
612 al. 2006, Alverson et al. 2007, Hoppenrath et al. 2007) and here they provided a good taxonomic
613 resolution for all the Thalassiosiraceae representatives except *T. gravida*, which shares identical 18S
614 rRNA gene with *T. rotula* (Fig. 1). These two species show low phylogenetic distances also on 28S
615 rRNA gene phylogeny (Fig. 2B) and can be correctly separated only after ITS sequencing
616 (Whittaker et al. 2012).

617 The 28S rRNA gene is a relatively good molecular marker to discriminate most of *Pseudo-*
618 *nitzschia* species although a better resolution of phylogenetic relationships can be generally
619 achieved with the ITS rRNA possibly supplemented by the analysis of the secondary structure of
620 the ITS2 (Lundholm et al. 2003, Amato et al. 2007, Lundholm et al. 2012, Lim et al. 2013, Orive et
621 al. 2013, Percopo et al. 2016). *Pseudo-nitzschia arctica* and *P. granii* share highly similar 18S
622 rRNA gene sequences (Fig. 1) but can be better discriminated based on 28S rRNA (Fig. 2A), ITS
623 and *rbcL* phylogenies (Percopo et al. 2016).

624 Similarly, the MALINA strains of *C. neogracilis* and two Arctic strains isolated from the
625 Greenland Sea (EU090013 and EU090014; Choi et al. 2008) share identical 18S rRNA sequences
626 (Fig. 1), but they are genetically different at both 28S and ITS levels (Figs. 2B, and 3). 28S and ITS
627 rRNA phylogenies consistently grouped sequences from the Arctic strains of *C. neogracilis* into
628 four phylogenetically discrete clades (Fig. 3). The differences in the ITS secondary structure
629 confirm this grouping and would indicate reproductive isolation between the four clades of *C.*
630 *neogracilis* which may correspond to closely related but distinct cryptic species. Specifically a CBC
631 in helix IIa (Fig. 9) suggests reproductive isolation between clade I and clade II vs. clade IV, and
632 similarly the presence of at least a Hemi-CBC in the Helix III between Clade I and Clade II, as well
633 as between Clade III and all the other clades, suggests that the different clades are unable to
634 interbreed (Coleman 2009). The secondary structures of both ITS-1 and ITS-2 are involved in

635 ribosome assembly (Tschochner & Hurt 2003) and changes in paired positions likely affects gamete
636 compatibility preventing cells differing by CBC or Hemi-CBC from mating (Coleman 2001). For
637 diatoms, inability to interbreed has been demonstrated between strains differing by CBC or Hemi-
638 CBC in the ITS-2 within the *P. pseudodelicatissima* species complex (Amato et al. 2007).

639 The sympatric occurrence of distinct genetic clades of *C. neogracilis* in the Beaufort Sea gives
640 further support to the hypothesis that they should be considered separate species unable to
641 interbreed rather than different genotypes of a single species. Closely-related species or genotypes
642 can co-occur in the same environment and similar results were found previously in dinoflagellates.
643 Several ITS genotypes from the Atama complex, which consisted of *Alexandrium tamarense*
644 (Lebour) Balech, *Alexandrium fundyense* Balech, and *Alexandrium catenella* (Whedon & Kofoid)
645 Balech, co-occurred in the Chukchi Sea (Gu et al. 2013). In contrast, the Arctic *Micromonas*
646 (Lovejoy et al. 2007, Balzano et al. 2012b) consisted in a single ITS genotype (Balzano et al.
647 2012a), which dominated both surface and DCM, waters throughout the Beaufort Sea during the
648 MALINA cruise (Balzano et al. 2012b).

649 Notably, clone libraries based on 18S rRNA gene sequences, and high throughput amplicon
650 sequencing of the V4 or V9 regions of the 18S rRNA, which are widely used in environmental
651 studies (Stoeck et al. 2010, Comeau et al. 2011, Logares et al. 2012, Logares et al. 2014, Balzano et
652 al. 2015), failed to discriminate among the four clades of *C. neogracilis* and recovered them as a
653 unique genotype (Pawlowski et al. 2008, Lovejoy & Potvin 2011).

654 Both 18S and 28S rRNA genes are too conserved for some genera failing to discriminate the
655 different species. For example, *A. septentrionalis* shared identical 18S rRNA and 28S rRNA gene
656 sequences with *A. longicornis* (Figs 1, and 2A). These two species can be distinguished only using a
657 combination of several nuclear and plastidial encoded genes (Sorhannus & Fox 2012).

658 The 18S rRNA gene is highly conserved also within the family Cymatosiraceae, where *A.*
659 *cornucervis* strain RCC2270 shares almost identical 18S rRNA with two GenBank sequences from
660 *M. polymorphus* (Fig. 1), and the 2 species share 100% identity in the V4 region (Luddington et al.

661 2012). The extent of the variability of the 28S rRNA gene within the Cymatosiraceae is not clear
662 since no other sequence from this family is available on GenBank and *A. cornucervis* RCC2270
663 shares highly similar 28S rRNA gene with two unidentified Cymatosiraceae strains (Fig. 2B).

664 Overall, ITS-2 provides a higher taxonomic resolution than 28S, but although it was proposed as
665 a universal barcode for diatoms (Moniz & Kaczmarska 2010, Guo et al. 2015), very few ITS
666 sequences are available to date in GenBank compared to 18S and 28S and its high variability makes
667 the alignment between different genera difficult or even impossible. Similarly the 28S rRNA gene
668 is less conserved than the 18S rRNA allowing a better discrimination between congeneric species
669 but 28S sequences are available for a larger number of diatom species. Ideally sequencing the entire
670 rRNA operon from the same specimen would allow the best taxonomic resolution and provide
671 taxonomic annotation from most species in environmental studies. Single molecule sequencing
672 technologies such as PacBio could allow the sequencing of reads as long as 5,000 bp (Mikheyev &
673 Tin 2014, Schloss et al. 2016). For current sequencing technologies the 28S rRNA seems the best
674 compromise between resolute power and easiness of alignment, for environmental studies focused
675 on diatoms, whereas 18S rRNA gene sequencing can be used for general studies on microbial
676 eukaryotes.

677 *Diatoms in the Beaufort Sea.* Diatoms represented an important fraction of the nano- and
678 microphytoplankton identified during the MALINA cruise (Balzano et al. 2012b, Coupel et al.
679 2015) with *Chaetoceros* and *Thalassiosira* being the most represented genera. Different species
680 from these two genera are frequently observed in Arctic waters where they typically dominate
681 phytoplankton assemblages (Booth & Horner 1997, Lovejoy et al. 2002, Ratkova & Wassmann
682 2002), eventually forming spring blooms (Booth et al. 2002, Sukhanova et al. 2009).

683 In spite of the high diversity reported in previous studies (Sukhanova et al. 2009), only few
684 environmental ribotypes associated with *T. nordenskiöldii* were detected by T-RFLP among sorted
685 photosynthetic eukaryotes during the MALINA cruise (Balzano et al. 2012b) and only *T.*
686 *nordenskiöldii*, *T. gravida*, *Thalassiosira pacifica* and few undetermined species were observed by

687 microscopy counts (<http://malina.obs-vlfr.fr>), accounting for a low proportion of the phytoplankton
688 community. Clearly, *Thalassiosira* species did not bloom in the Beaufort Sea during late summer
689 2009 and *T. gravida*, *T. cf. hispida*, and *T. minima* were possibly only present in low abundance.

690 The high number of *Chaetoceros* strains (45), mostly represented by *C. gelidus* and *C.*
691 *neogracilis*, reflected the dominance of these two species in the summer phytoplankton
692 assemblages, already shown by the genetic libraries (Balzano et al. 2012b). Notably, phytoplankton
693 counts confirmed the high abundance of *C. gelidus* and other unidentified morphotypes, but barely
694 reported the occurrence of *C. neogracilis*. This discrepancy indicates that cells of *C. neogracilis*
695 might have been erroneously attributed to several different solitary species, such as *C. tenuissimus*
696 or *Chaetoceros simplex* Ostenfeld, or other undetermined *Chaetoceros*. We also suggest that cell
697 chains of *C. neogracilis*, which were described for the first time in this study, might have been
698 wrongly identified as the freshwater species *Chaetoceros wighamii* Brightwell ([http://malina.obs-](http://malina.obs-vlfr.fr)
699 [vlfr.fr](http://malina.obs-vlfr.fr); see Bosak et al. 2015 for a discussion on *C. wighamii*). Similarly, the doubtful reports of *C.*
700 *wighamii* from the Baltic Sea and Danish waters could indeed refer to *C. neogracilis*, as suggested
701 by the morphological and ultrastructural similarity between Arctic strains of *C. neogracilis*
702 described in this study and culture material from Danish waters attributed to *C. wighamii* (see fig.
703 224 in Jensen & Moestrup 1998).

704 Other colonial *Chaetoceros* species found in the phytoplankton counts were not isolated in this
705 study because they might be more difficult to bring into culture compared to *C. gelidus* and *C.*
706 *neogracilis*, or because they are rare, as suggested by their absence in the 18S rRNA libraries and in
707 T-RFLP analyses (Balzano et al. 2012b).

708 Interestingly most of the *C. neogracilis* strains from Clade I and Clade II as well as all the strains
709 of Clade IV were isolated from surface waters (Table 1), whereas 5 out of 8 strains of *C. gelidus*
710 and both *C. neogracilis* Clade III strains were isolated from DCM waters. During the MALINA
711 cruise surface waters were warmer, less saline (Table S1), and poorer in nutrients
712 (<http://malina.obs-vlfr.fr/data.html>) compared to DCM waters. We do not know whether these

713 patterns are indicative of ecological preferences for these genotypes. However surface genotypes
714 might be adapted to lower salinities, higher irradiation, higher temperatures and lower nutrient
715 concentrations. Unfortunately, the different clades of *C. neogracilis* have identical T-RFLP
716 ribotypes and therefore their relative contribution to the environmental samples from the MALINA
717 cruise (Balzano et al. 2012b) cannot be discerned.

718 Notably, some of the strains isolated here show similarities with specimens from other
719 environments affected by seasonal salinity shifts similar to those characterizing the Beaufort Sea.
720 One of the *C. neogracilis* strains belonging to Clade II, CPH9 (Fig. 3A), was isolated in the Baltic
721 Sea, and *C. closterium* RCC1985 forms a clade, in the 28S rRNA tree, with a strain (K-520, Fig.
722 2A) which has been isolated from Kattegat (Lundholm et al. 2002). Interestingly, a number of
723 environmental sequences as well as photosynthetic flagellates isolated from the surface waters of
724 the Beaufort Sea during the MALINA cruise are genetically related to strains or environmental
725 sequences from the Baltic Sea (Balzano et al. 2012a, Balzano et al. 2012b). Despite the significant
726 differences in temperature and salinity between the Beaufort Sea and both the Baltic Sea and the
727 Kattegat, the genetic similarities found in samples from these areas might be associated with the
728 seasonal ice and the shifts in salinity occurring in these environments.

729 *The Chaetoceros neogracilis species complex.* *Chaetoceros neogracilis* was originally described
730 as *Chaetoceros gracile* Schütt from the Baltic Sea as solitary, small *Chaetoceros* species (Schütt
731 1895). Due to the scanty original description and to the lack of distinctive features in such small
732 single cell-taxa, the name has most probably been attributed to different and not related taxa
733 collected worldwide (Rines & Hargraves 1988). All the Arctic strains isolated during the MALINA
734 cruise share a similar cell morphology with *C. neogracilis*, together with a prevalent absence of
735 colony formation. Indeed, *C. neogracilis* was originally described as a solitary species whereas
736 some of the MALINA strains have been observed forming short colonies. Notably, the ability to
737 occasionally form colonies is common to other *Chaetoceros* species considered solitary, as it has
738 also been observed in the related species *C. tenuissimus* (D. Sarno, pers. observation). The original

739 description of the species (Schütt 1895) includes a spiny spore that unfortunately has not been
740 observed in our study.

741 Based on the available information, it is not possible to provide the authoritative taxonomic
742 revision required by the International Code of Nomenclature for algae, fungi, and plants (McNeill et
743 al. 2012) to establish each of the four clades as valid species and to assess if one of them
744 corresponds to *C. neogracilis* sensu stricto. Further analyses are required to provide additional
745 ultrastructural information on a larger number of strains from the four clades to be compared with
746 the type material of *C. neogracilis* and eventually designate an epitype. In the meantime, we
747 propose that the Arctic *Chaetoceros* strains sharing very similar morphology and molecular
748 signatures described here are considered as affiliated to *C. neogracilis* species complex. The
749 provisional ascription of the name *C. neogracilis* to the Arctic *Chaetoceros* complex is supported
750 by the fact that one of the strains (i.e., CPH9, syn K-1665, <http://www.sccap.dk/>) belonging to
751 Clade II of the species complex, was isolated from Danish waters in the Baltic Sea, which is the
752 type locality of *C. neogracilis*. The morphologically similar Antarctic species, which has been
753 frequently identified as *C. neogracilis* and is represented in this study by the strains AnM0002,
754 CCMP187, CCMP189 and CCMP190 (Choi et al. 2008), corresponds to a related but genetically
755 distinct (Figs. 1, 2B, and 7) and probably undescribed species, here named as *Chaetoceros* sp.

756 *Biogeography of Arctic diatoms.* Most of the diatom species (10 out of 17) characterized in this
757 study have a distribution confined to the northern/polar area, including *Pseudo-nitzschia arctica*
758 (Percopo et al. 2016), and the *C. neogracilis* species complex, which was one of the few Arctic
759 phylogenotypes identified by their 18S rRNA gene (Lovejoy & Potvin 2011) (Table 2). In addition, the
760 MALINA strain of *C. closterium* (RCC1985) is phylogenetically distant from any lineage described
761 for this species complex (Haitao et al. 2007) and might correspond to an Arctic genotype.

762 Endemism has been recently suggested for a number of Arctic protists from the Baffin Bay and the
763 Beaufort Sea (Terrado et al. 2013). Endemic polar species include in particular the green alga Arctic
764 *Micromonas* (Lovejoy et al. 2007), several foraminiferan species (Darling et al. 2007, Pawlowski et

765 al. 2008), and the Antarctic terrestrial diatoms *Pinnularia borealis* Ehrenberg and *Hantzschia*
766 *amphioxys* (Ehrenberg) Grunow (Souffreau et al. 2013).

767 Two species found here, *Porosira glacialis* and *T. gravida*, are considered to have bipolar
768 distribution (McMinn et al. 2005, Whittaker et al. 2012, Goes et al. 2014). The presence of the same
769 species in ecologically related but geographically distant environments, such as the Arctic and the
770 Antarctic, has been suggested for two *Fragilariopsis* Hustedt species (Lundholm & Hasle 2008) as
771 well as the dinoflagellate *Polarella glacialis* Montresor, Procaccini & Stoecker (Montresor et al.
772 2003) and the ciliate *Euplotes nobilii* Valbonesi & Luporini (Di Giuseppe et al. 2014). Polar species
773 can hardly survive in temperate and tropical waters and the evolution of polar species is thus
774 unlikely to arise from transport of living cells between Arctic and Antarctic waters. The presence of
775 bipolar species could be associated with a migration occurred during the last glacial period, where
776 colder seawater at low latitudes would have permitted the survival of cells during their transport
777 across the globe or due to more recent transport of resting forms (Montresor et al. 2003). Such
778 resting forms could survive tropical waters or in alternative they might have been transported across
779 the globe via the global ocean conveyor belt or other deep cold currents.

780 Few (5) of the strains characterized in this study belong to species that are supposed to have a
781 wide geographical distribution (Table 2). Molecular methods have demonstrated conspecificity in
782 widely distributed morphospecies, as for example some *Pseudo-nitzschia* (Lelong et al. 2012) or
783 *Skeletonema* (Kooistra et al. 2008) species. Other studies on plankton biogeography indicate that
784 populations previously thought to make up unique cosmopolitan species are often genetically
785 distinct and reproductively isolated (Kooistra et al. 2008, Casteleyn et al. 2010). Indeed, the
786 northern/polar ecotype of the worldwide-considered species, *C. socialis*, has been recently
787 described as a distinct species, i.e., *C. gelidus*, based on physiological, morphological and molecular
788 evidence (Degerlund et al. 2012, Huseby et al. 2012, Chamnansin et al. 2013). Subsequently all
789 the previous reports of *C. socialis* in Arctic waters (Booth et al. 2002, Ratkova & Wassmann 2002,

790 Sukhanova et al. 2009), including those reported for the MALINA cruise (Balzano et al. 2012b), are
791 likely to correspond to *C. gelidus*.

792 Similarly, the degree of interspecific divergence between the cosmopolitan *T. rotula* and the
793 bipolar *T. gravida* advocates they should be treated as separate species (Whittaker et al. 2012),
794 despite previous studies suggesting that the two morphotypes are likely to be a single species
795 (Syvertsen 1977, Sar et al. 2011). We cannot exclude that the use of more sensitive molecular
796 markers would allow to identify differences among geographical populations of bipolar or
797 cosmopolitan species, as demonstrated for the cosmopolitan species *Pseudo-nitzschia pungens*
798 (Casteleyn et al. 2010). Further analyses will be required to evaluate the slight difference here found
799 among the 28S rRNA gene sequences of the Arctic and Antarctic strains of *Porosira glacialis*.

800 Therefore while some species distribution patterns seem to support the hypothesis of ubiquity
801 (Finlay & Fenchel 2004), other species are far more restricted. The availability of validated
802 reference sequences for arctic diatoms will facilitate the interpretation of metabarcoding data and
803 will allow to test theories on dispersal and biogeographic patterns in protists using large scale
804 screening of environmental samples.

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806

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Table 1. List of the strain isolated during the MALINA cruise and used in the present study.

Family	Species	Strain code ¹	Isolation site ²		Morphology ³	Genbank accession numbers ⁴		
			Station	Depth (m)		18S	28S	ITS
Bacillariaceae	<i>Cylindrotheca closterium</i>	RCC1985	280	30	LM, TEM	JF794039	JQ995403	
	<i>Nitzschia pellucida</i>	RCC2276	BEA130709A	0	LM, TEM	JF794052	JQ995450	
	<i>Pseudo-nitzschia granii</i>	RCC2006	PAC080709A	5	LM, TEM		JQ995420	
	<i>Pseudo-nitzschia granii</i>	RCC2008	PAC080709A	5	LM	JN934671	JQ995421	
	<i>Pseudo-nitzschia granii</i>	RCC2273	PAC060709A	0			JQ995391	
	<i>Pseudo-nitzschia arctica</i>	RCC2002	690	29	LM, TEM, SEM		JQ995416	
	<i>Pseudo-nitzschia arctica</i>	RCC2004	690	29	LM, TEM, SEM	JF794046	JQ995418	
	<i>Pseudo-nitzschia arctica</i>	RCC2005	690	29	LM, TEM		JQ995419	
	<i>Pseudo-nitzschia arctica</i>	RCC2517	690	29	LM, TEM, SEM		JQ995461	
Fragilariaceae	<i>Synedropsis hyperborea</i>	RCC2043	280	30	LM, TEM, SEM	JF794051	JQ995434	
	<i>Synedropsis hyperborea</i>	RCC2520	280	30	LM		JQ995463	
Attheyaceae	<i>Attheya septentrionalis</i>	RCC1986	280	30	LM, TEM, SEM	JF794040	JQ995404	
	<i>Attheya septentrionalis</i>	RCC1988	280	30	LM		JQ995405	
	<i>Attheya septentrionalis</i>	RCC2042	680	3	LM	JN934675	JQ995433	
Thalassiosiraceae	<i>Thalassiosira gravida</i>	RCC1984	280	30	LM	JN934669	JQ995402	
	<i>Thalassiosira gravida</i>	RCC1999	280	30	LM, TEM, SEM		JQ995414	
	<i>Thalassiosira cf. hispida</i>	RCC2521	680	40	TEM, SEM	JN934691	JQ995464	
	<i>Thalassiosira minima</i>	RCC2265	394	3	LM, TEM, SEM	JN934676	JQ995440	
	<i>Thalassiosira minima</i>	RCC2266	394	3	LM, TEM, SEM		JQ995441	
	<i>Thalassiosira minima</i>	RCC2269	PAC050709A	0	LM, TEM, SEM		JQ995444	
	<i>Thalassiosira nordenskiöldii</i>	RCC2000	690	29	LM, TEM, SEM	JF794045	JQ995415	
	<i>Thalassiosira nordenskiöldii</i>	RCC2021	680	3	LM, TEM, SEM		JQ995428	
	<i>Thalassiosira nordenskiöldii</i>	RCC2522	620	3	LM			
	<i>Porosira glacialis</i>	RCC1995	690	29	LM, SEM, TEM			
	<i>Porosira glacialis</i>	RCC2039	690	29	LM	JN934673	JQ995432	
	<i>Shionodiscus bioculatus</i>	RCC1991	620	65	LM, TEM, SEM	JF794041	JQ995408	
	Cymatosiraceae	<i>Arcocellulus cornucervis</i>	RCC2270	ARC120709A	0	LM, SEM, TEM	JN934677	JQ995445

Hemiaulaceae	<i>Eucampia groenlandica</i>	RCC1996	690	29	LM, TEM, SEM	JF794043	JQ995412	
	<i>Eucampia groenlandica</i>	RCC2037	690	29	LM, SEM		JQ995430	
	<i>Eucampia groenlandica</i>	RCC2038	690	29	LM, SEM		JQ995431	
Chaetoceraceae	<i>Chaetoceros decipiens</i>	RCC1997	690	29	LM, TEM, SEM	JF794044	JQ995413	
	<i>Chaetoceros gelidus</i>	RCC1990	620	65	LM		JQ995407	
	<i>Chaetoceros gelidus</i>	RCC1992	620	65	LM, TEM, SEM	JF794042	JQ995409	
	<i>Chaetoceros gelidus</i>	RCC1994	690	29	LM, SEM		JQ995411	
	<i>Chaetoceros gelidus</i>	RCC2046	280	30	LM		JQ995435	
	<i>Chaetoceros gelidus</i>	RCC2271	690	3	LM,SEM		JQ995446	
	<i>Chaetoceros gelidus</i>	MALINA E65 PG4	690	29			JQ995393	
	<i>Chaetoceros gelidus</i>	MALINA E65 PG18	690	29				
	<i>Chaetoceros gelidus</i>	MALINA S135	BEA140709A	0			JQ995396	
	<i>Chaetoceros neogracilis</i> clade I	RCC2003	690	29	LM		JQ995417	KT860511
	<i>Chaetoceros neogracilis</i> clade I	RCC2011	620	3	LM		JQ995423	KT860513
	<i>Chaetoceros neogracilis</i> clade I	RCC2017	760	3	LM, TEM		JQ995427	KT860517
	<i>Chaetoceros neogracilis</i> clade I	RCC2262	460	3	LM		JQ995437	KT860520
	<i>Chaetoceros neogracilis</i> clade I	RCC2263	235	3	LM		JQ995438	KT860521
	<i>Chaetoceros neogracilis</i> clade I	RCC2264	235	3	LM		JQ995439	KT860522
	<i>Chaetoceros neogracilis</i> clade I	RCC2267	394	3			JQ995442	KT860523
	<i>Chaetoceros neogracilis</i> clade I	RCC2274	620	3	LM		JQ995448	KT860526
	<i>Chaetoceros neogracilis</i> clade I	RCC2275	620	3	LM		JQ995449	KT860527
	<i>Chaetoceros neogracilis</i> clade I	RCC2278	320	3	LM		JQ995452	KT860529
	<i>Chaetoceros neogracilis</i> clade I	RCC2279	320	3	LM		JQ995453	KT860530
	<i>Chaetoceros neogracilis</i> clade I	RCC2280	760	3	LM		JQ995454	KT860531
	<i>Chaetoceros neogracilis</i> clade I	RCC2281	760	3	LM		JQ995455	KT860532
	<i>Chaetoceros neogracilis</i> clade I	RCC2507	235	25	LM		JQ995459	KT860536
	<i>Chaetoceros neogracilis</i> clade I	MALINA S441 P21-E6	320	3			JQ995397	KT860541
	<i>Chaetoceros neogracilis</i> clade I	MALINA S502 P27.B3	760	3			JQ995399	KT860540
	<i>Chaetoceros neogracilis</i> clade I	MALINA S509	760	3			KT884482	KT884482
	<i>Chaetoceros neogracilis</i> clade I	MALINA S510	760	3			KT884483	KT884483

<i>Chaetoceros neogracilis</i> clade I	MALINA S511	760	3			KT884484	KT884484
<i>Chaetoceros neogracilis</i> clade I	MALINA S512	760	3			KT884485	KT884485
<i>Chaetoceros neogracilis</i> clade II	RCC2261	460	3	LM		JQ995436	KT860519
<i>Chaetoceros neogracilis</i> clade II	RCC2268	BEA130709A	0	LM		JQ995443	KT860524
<i>Chaetoceros neogracilis</i> clade II	RCC2272	BEA130709A	0	LM,SEM		JQ995447	KT860525
<i>Chaetoceros neogracilis</i> clade II	RCC2277	BEA130709A	0	LM		JQ995451	KT860528
<i>Chaetoceros neogracilis</i> clade II	RCC2282	760	3	LM		JQ995456	KT860533
<i>Chaetoceros neogracilis</i> clade II	RCC2318	620	65	LM	JN934684	JQ995457	KT860534
<i>Chaetoceros neogracilis</i> clade II	RCC2506	235	3	LM		JQ995458	KT860535
<i>Chaetoceros neogracilis</i> clade II	MALINA E43.N2	BEA140709A	0			JQ995392	
<i>Chaetoceros neogracilis</i> clade III	RCC1989	620	65	LM		JQ995406	KT860509
<i>Chaetoceros neogracilis</i> clade III	RCC1993	620	65	LM		JQ995410	KT860510
<i>Chaetoceros neogracilis</i> clade IV	RCC2010	620	3	LM, SEM		JQ995422	KT860512
<i>Chaetoceros neogracilis</i> clade IV	RCC2012	110	3	LM, SEM,TEM		JQ995424	KT860514
<i>Chaetoceros neogracilis</i> clade IV	RCC2014	110	3	LM, TEM		JQ995425	KT860515
<i>Chaetoceros neogracilis</i> clade IV	RCC2016	760	3	LM, SEM	JF794049	JQ995426	KT860516
<i>Chaetoceros neogracilis</i> clade IV	RCC2022	680	3	LM, SEM		JQ995429	KT860518
<i>Chaetoceros neogracilis</i> clade IV	MALINA FT56.6 PG6	110	3			JQ995395	KT860542

¹ RCC: Roscoff culture collection. More information on the strains is available at <http://roscoff-culture-collection.org/>. Strains without an RCC code are no longer available.

² Sampling location of the MALINA cruise. See Table S1 for more details

³ Technique used for the morphological identification: LM, Light Microscopy; TEM, Transmission Electron Microscopy; SEM, Scanning Electron Microscopy.

⁴ Please note that the V4 region of the 18S rRNA gene has been sequenced from all the strains.

Table 2. Geographic distribution and morphological references of the species identified in the present study.

Species	Morphological references	Global distribution	Distribution in Arctic waters
<i>Cylindrotheca closterium</i> (Ehrenberg) Lewin & Reimann	Hasle & Syvertsen 1997, and references therein Jahn & Kusber 2005	Cosmopolitan (Hasle & Syvertsen 1997) Common in Arctic waters	Beaufort Sea (Horner & Schrader 1982) Chukchi Sea (von Quillfeldt 2000) White and Barents Sea (Luddington et al. 2016) Laptev Sea (Tuschling et al. 2000) Central Arctic Ocean (Katsuki et al. 2009)
<i>Nitzschia pellucida</i> Grunow	Bérard-Therriault et al. 1999, and references therein	Northern cold water region (Bérard-Therriault et al. 1999) Antarctica (Hällfors 2004) European freshwater environments (Cărăus 2012) Ishigaki Island, Japan (Lundholm et al. 2002)	Chukchi Sea (von Quillfeldt 2000)
<i>Pseudo-nitzschia granii</i> (Hasle) Hasle	Hasle & Syvertsen 1997, and references therein Marchetti et al. 2008	Northern cold water region to temperate? (Hasle & Syvertsen 1997) Northern Atlantic (Hasle 1964) Subarctic Pacific (Marchetti et al. 2008, this study)	Norwegian Sea (Hasle 1964) Chukchi Sea (von Quillfeldt et al. 2003) White and Barents Seas (Luddington et al. 2016)
<i>Pseudo-nitzschia arctica</i> Percopo & Sarno	Percopo et al. 2016	Recently described from Arctic waters (Percopo et al. 2016)	Beaufort Sea, Barrow Strait, Baffin Bay (Percopo et al. 2016)
<i>Synedropsis hyperborea</i> (Grunow) Hasle, Medlin & Syvertsen	Hasle et al. 1994	Northern cold water region (Hasle & Syvertsen 1997) Common in Arctic waters (Hasle et al. 1994)	Frobisher Bay, Greenland, Barents Sea (Hasle et al. 1994) Chukchi Sea (von Quillfeldt et al. 2003)
<i>Attheya septentrionalis</i> (Østrup) Crawford	Crawford et al. 1994 Stonik et al. 2006	Northern cold water region to temperate (Hasle & Syvertsen 1997) Common in Arctic waters	Nansen Basin (Gosselin et al. 1997) Chukchi Sea (von Quillfeldt et al. 2003)

			Baffin Bay (Caron et al. 2004) White and Barents Sea (Luddington et al. 2016) Laptev Sea (Tuschling et al. 2000)
<i>Thalassiosira gravida</i> Cleve	Syvertsen 1977	Northern and southern cold water regions (Whittaker et al. 2012) Common in Arctic waters	Nansen Basin, Chukchi Sea (Gosselin et al. 1997) Baffin Bay (Lovejoy et al. 2002) Laptev Sea (Tuschling et al. 2000) Central Arctic Ocean (Katsuki et al. 2009)
<i>Thalassiosira cf. hispida</i> Syvertsen	Syvertsen 1986	Northern cold water region to temperate (Hasle & Syvertsen 1997)	Amundsen Gulf (Luddington et al. 2016) Central Arctic Ocean (Katsuki et al. 2009) Svalbard and the Barents Sea (von Quillfeldt 2000) Chukchi Sea (von Quillfeldt et al. 2003)
<i>Thalassiosira minima</i> Gaarder	Hasle & Syvertsen 1997, and references therein Hoppenrath et al. 2007	Cosmopolitan excluding polar regions (Hasle & Syvertsen 1997) North Sea (Hoppenrath et al. 2007) North Atlantic Ocean (Luddington et al. 2016, as <i>T. aff. minima</i>) Warm waters in coastal and estuarine systems (Guinder et al. 2012)	First report in this study
<i>Thalassiosira nordenskiöldii</i> Cleve	Hasle & Syvertsen 1997, and references therein	Northern cold water region to temperate (Hasle & Syvertsen 1997) Common in Arctic waters	Amundsen Gulf (Luddington et al. 2016) Canadian Arctic (Aizawa et al. 2005) Baffin Bay (Caron et al. 2004) Barents Sea (Degerlund & Eilertsen 2010) Laptev Sea (Tuschling et al. 2000) Chukchi Sea (von Quillfeldt et al.

			2003) Central Arctic Ocean (Katsuki et al. 2009)
<i>Porosira glacialis</i> (Grunow) Jørgensen	Hasle & Syvertsen 1997, and references therein	Northern cold water region to temperate, southern cold water region (Hasle & Syvertsen 1997) Common in Arctic waters	Amundsen Gulf (Luddington et al. 2016) Chukchi Sea (Gosselin et al. 1997) Beaufort Sea (Sukhanova et al. 2009) White and Barents Seas (Olli et al. 2002) Central Arctic Ocean (Katsuki et al. 2009)
<i>Shionodiscus bioculatus</i> (Grunow) Alverson, Kang & Theriot	as <i>Thalassiosira bioculata</i> : Hasle & Syvertsen 1997, and references therein Bérard-Therriault et al. 1999, and references therein	Northern cold water region (Hasle & Syvertsen 1997) Common in Arctic waters	Amundsen Gulf (Luddington et al. 2016) Chukchi Sea (von Quillfeldt et al. 2003) White and Barents Sea (Luddington et al. 2016) Norwegian coastal waters (Degerlund & Eilertsen 2010) Baffin Bay (Booth et al. 2002) Central Arctic Ocean (Katsuki et al. 2009)
<i>Arcocellulus cornucervis</i> Hasle, von Stosch & Syvertsen	Hasle et al. 1983	Northern cold and temperate waters, New Zealand (Hasle & Syvertsen 1997) Mediterranean Sea (Percopo et al. 2011)	Baffin Bay (Lovejoy et al. 2002)
<i>Eucampia groenlandica</i> Cleve	Syvertsen & Hasle 1983	Northern cold water region (Hasle & Syvertsen 1997) Common in Arctic waters	Baffin Bay (Cleve 1896) Laptev Sea (Tuschling et al. 2000) Barents Sea (Luddington et al. 2016)
<i>Chaetoceros decipiens</i> Cleve	Hasle & Syvertsen 1997, and references therein Jensen & Moestrup 1998	Cosmopolitan (Hasle & Syvertsen 1997) Common in Arctic waters	North Pacific and Bering Sea (Aizawa et al. 2005) Baffin Bay (Caron et al. 2004) Barents Sea (Ratkova & Wassmann 2002)

			Norwegian coastal waters (Degerlund & Eilertsen 2010)
<i>Chaetoceros gelidus</i> Chamnansinp, Li, Lundholm & Moestrup	Chamnansinp et al. 2013	Northern cold water region (Chamnansinp et al. 2013)	Barents Sea, Norwegian Sea, Greenland (Chamnansinp et al. 2013)
<i>Chaetoceros neogracilis</i> (Schütt) VanLandingham	Schütt 1895 (as <i>C. gracile</i>) See discussion	Baltic Sea (Hällfors 2004, Majaneva et al. 2012)	Svalbard (Choi et al. 2008) Beaufort Sea (Lovejoy & Potvin 2011)

FIGURE LEGEND

Figure 1. Full 18S rRNA phylogenetic tree derived from Maximum Likelihood (ML) analysis. The tree includes at least one sequence from each genotype found within the diatom strains isolated during the MALINA cruise. Four sequences from radial centrics (*Corethron hystrix*, *Corethron pennatum*, *Rhizosolenia setigera*, and *Rhizosolenia similoides*) have been used as outgroup. The MALINA strains sequenced here are labelled in bold whereas other strains isolated from Arctic waters are underlined. The Genbank accession number is indicated next to the strain code. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches from left (ML) to right (Neighbour-joining). Missing percentage values and “_” indicate that bootstrap values < 50 % were obtained for the corresponding node. Asterisks indicate strains isolated from the North Pacific Ocean.

Figure 2. 28S rRNA phylogenetic tree inferred by maximum likelihood (ML) analysis for the (A) pennate and (B) centric diatoms isolated during the MALINA cruise. The MALINA strains sequenced here are labelled in bold whereas other strains isolated from Arctic waters are underlined. The evolutionary histories were inferred using maximum likelihood. The percentage of trees in which the associated taxa cluster together is shown next to the branches based on Maximum Likelihood (left) and Neighbour joining (right). ML and NJ values are indicated next to the branch nodes as described in Fig. 1. Asterisks indicate strains isolated from the North Pacific Ocean.

Figure 3. 28S rRNA (A), ITS-1 (B), and 5.8S+ITS-2 (C) phylogenetic trees for the strains of *Chaetoceros neogracilis* strains isolated from the Beaufort Sea. For the 28S, *C. gelidus* was used to root the phylogenetic tree whereas for the ITS-1 and 5.8S + ITS-2 trees, the Antarctic strains of *Chaetoceros* sp. (CCMP187, CCMP189, CCMP190) were used as outgroup. The bootstrap values are indicated next to the branches as for Figure 1.

Figure 4. (A) *Cylindrotheca closterium*: TEM micrograph, RCC1985. Detail of the valve in which is visible the raphe interruption. Scale bar, 2 μm . (B-D) *Nitzschia pellucida*: (B) TEM micrograph, RCC2276. Whole valve. Scale bar, 5 μm . (C) TEM micrograph, RCC2276. Detail of the valve. Note the central larger interspace. Scale bar, 2 μm . (D) TEM micrograph, RCC2276. Detail of cell apex. Scale bar, 2 μm . (E-G) *Pseudo-nitzschia granii*: (E) TEM micrograph, RCC2006. Whole valve. Scale bar, 5 μm . (F) TEM micrograph, RCC2006. Detail of the valve. Note the few incomplete poroids (arrows). Scale bar, 0.5 μm . (G) TEM micrograph, RCC2006. Detail of the valve with scattered complete poroids. Scale bar, 1 μm . (H-I) *Pseudo-nitzschia arctica*: (H) LM micrograph, RCC2002. A colony of two cells in girdle view. Scale bar, 20 μm . (I) TEM micrograph, RCC2004. Detail of the valve. Note the central larger interspace. Scale bar, 1 μm . (J-N) *Synedropsis hyperborea*: (J) LM micrograph, RCC2043. Cell in valve view. Scale bar, 2 μm . (K) SEM micrograph, RCC2043. External view of the central part of the valve. Scale bar, 1 μm . (L) SEM micrograph, RCC2043. Internal view of the apex. Note apical slit field and rimoportula. Scale bar, 0.1 μm . (M) SEM micrograph, RCC2043. External view of the apex. Note apical slit field and absence of rimoportula. Scale bar, 0.5 μm . (N) SEM micrograph, RCC2043. External view of the apex. Note apical slit field and rimoportula. Scale bar, 0.2 μm .

Figure 5. (A-E) *Attheya septentrionalis*: (A) LM micrograph, RCC1986. Cells in girdle view. Scale bar, 10 μm . (B) LM micrograph, RCC2042. A cell in girdle view. Scale bar, 20 μm . (C) TEM micrograph, RCC1986. A circular valve. Scale bar, 1 μm . (D) TEM micrograph, RCC2042. A horn with three longitudinal strips. Scale bar, 0.5 μm . (E) TEM micrograph, RCC1986. A horn with four longitudinal strips. Scale bar, 0.5 μm . (F-G) *Thalassiosira gravida*: (F) LM micrograph, RCC1999. Three cells joined in colony. Scale bar, 20 μm . (G) TEM micrograph, RCC1999. A valve with the central cluster of fultoportulae and several fultoportulae scattered on the valve face. Scale bar, 5 μm . (H-K) *Thalassiosira cf. hispida*: (H) SEM micrograph, RCC2521. A cell in valve view with a ring of marginal fultoportulae and one central fultoportula. Note the rimoportula between two marginal fultoportulae. Scale bar, 1 μm . (I) TEM micrograph, RCC2521. Detail of a valve; short and minute spines are present on the hyaline margin and in the areolae foramina. Scale bar, 1 μm . (J) SEM micrograph, RCC2521. The girdle composed by the valvocopula, a copula and open bands. Scale bar, 1 μm . (K) TEM micrograph, RCC2521. Detail of the fultoportula. Scale bar, 0.2 μm . (L-P) *Thalassiosira minima*: (L) LM micrograph, RCC2269. Cell in girdle view with two chloroplasts. Scale bar, 2 μm . (M) SEM micrograph, RCC2266. External view of the valve with a ring of marginal fultoportulae and two central fultoportulae. Note the rimoportula between two marginal fultoportulae. Scale bar, 1 μm . (N) SEM micrograph, RCC2269. Internal view of a valve. Scale bar, 1 μm . (O) TEM micrograph, RCC2266. Five central fultoportulae. Scale bar, 1 μm . (P) SEM micrograph,

RCC2266. Detail of two marginal fuloportulae with the small external labiate-shaped protrusions on the external face of the valve. Scale bar, 1 μm .

Figure 6. (A-C) *Thalassiosira nordenskiöldii*: (A) LM micrograph, RCC2000. A colony in girdle view. Scale bar, 5 μm . (B) TEM micrograph, RCC2000. A valve with a marginal ring of fuloportulae, one central fuloportula and one rimoportula positioned within two marginal fuloportulae. Scale bar, 2 μm . (C) SEM micrograph, RCC2000. A cell with ring of fuloportulae with long external tubes bearing a terminal collar. Scale bar, 1 μm . (D-F) *Porosira glacialis*: (D) LM micrograph, RCC1995. Cell in girdle view. Scale bar, 10 μm . (E) LM micrograph, RCC1995. Cell in valve view. Scale bar, 10 μm . (F) TEM micrograph, RCC1995. A valve with numerous fuloportulae scattered over the valve surface. Note the central annulus and the marginal rimoportula (arrow). Scale bar, 10 μm . (G-I) *Shionodiscus bioculatus*: (G) LM micrograph, RCC1991. A cell in girdle view. Scale bar, 20 μm . (H) SEM micrograph, RCC1991. External view of a cell; note the marginal ring of fuloportulae, the single fuloportula in the valve centre and a subcentral rimoportula. Scale bar, 10 μm . (I) TEM micrograph, RCC1991. Whole valve. Scale bar, 10 μm . (J-L) *Arcocellulus cornucervis*: (J) SEM micrograph, RCC2270. A slightly curved cell in girdle view. Note the conspicuous branches of the pili (arrow). Scale bar, 1 μm . (K) SEM micrograph, RCC2270. A pili valve (left) and a process valve (right). Note the ocelluli (arrows). Scale bar, 1 μm . (L) A pili valve in which the short spinules are visible near the pilus base (arrows). Scale bar, 2 μm .

Figure 7. (A) *Arcocellulus cornucervis*: TEM micrograph, RCC2270. A process valve in which the two ocelluli are visible (arrows). Scale bar, 1 μm . (B-C) *Eucampia groenlandica*: (B) LM micrograph, RCC2037. Part of a colony. Scale bar, 5 μm (C) SEM micrograph, RCC2037. A valve with the central rimoportula (arrow). Scale bar, 1 μm . (D-I) *Chaetoceros decipiens*: (D) LM micrograph, RCC1997. Part of a colony. Scale bar, 20 μm . (E) LM micrograph, RCC1997. A solitary cell. Scale bar, 20 μm . (F) TEM micrograph, RCC1997. A terminal valve. Scale bar, 5 μm . (G) SEM micrograph, RCC1997. Two intercalary valves. Scale bar, 1 μm . (H) TEM micrograph, RCC1997. A terminal valve. Note the central process. Scale bar, 5 μm . (I) TEM micrograph, RCC1997. A girdle band with parallel costae and small poroids. Scale bar, 5 μm . (J-L) *Chaetoceros gelidus*: (J) LM micrograph, RCC2271. A curved chain. Note the two straight setae on the upper part of the picture. Scale bar, 20 μm . (K) LM micrograph, RCC2271. A spherical colony. Scale bar, 50 μm . (L) SEM micrograph, RCC2271. Two intercalary valves with the narrow aperture. Scale bar, 1 μm .

Figure 8. (A-C) *Chaetoceros gelidus*: (A) TEM micrograph, RCC2271. Intercalary valve. Scale bar, 1 μm . (B) SEM micrograph, RCC2271. Detail of the two types of setae, the short (on the upper part of the picture) and the straight long seta (crossing the picture). Note the absence of spines in a large part of the long seta. Scale bar, 5 μm . (C) SEM micrograph, RCC2271. A spore. Scale bar, 1 μm . (D-N) *Chaetoceros neogracilis*: (D) LM micrograph, RCC2272. A solitary cell. Scale bar, 10 μm . (E) LM micrograph, RCC2017. A solitary cell. Scale bar, 10 μm . (F) LM micrograph, RCC2016. A solitary cell. Scale bar, 10 μm . (G) LM micrograph, RCC1989. A colony of four cells. Scale bar, 5 μm . (H) SEM micrograph, RCC2012. Detail of a colony. Note quite narrow apertures. Scale bar, 1 μm . (I) SEM micrograph, RCC2012. Terminal valve with

the external tube. Scale bar, 2 μm . (J) TEM micrograph, RCC2012. Terminal valve with the central slit-like process. Scale bar, 2 μm . (K) TEM micrograph, RCC2012. Intercalary valve. Scale bar, 2 μm . (L) TEM micrograph, RCC2271, Intercalary valve. Scale bar, 2 μm . (M) SEM micrograph, RCC2271. Setae with arrowhead-shaped spines. Scale bar, 1 μm . (N) SEM micrograph, RCC2271. Detail of a seta with spines and long spiral costae interconnected by short transverse costae. Scale bar, 0.5 μm .

Figure 9. FIG. 9. Diagrams of the secondary structure of the ITS-2 transcripts of *Chaetoceros neogracilis* Clade I RCC2279. The boxes indicate the structural variations found in *C. neogracilis* Clade I with respect to the other clades. Nucleotides which differ between *C. neogracilis* Clade I and the other three clades are marked with black background.

Supporting information

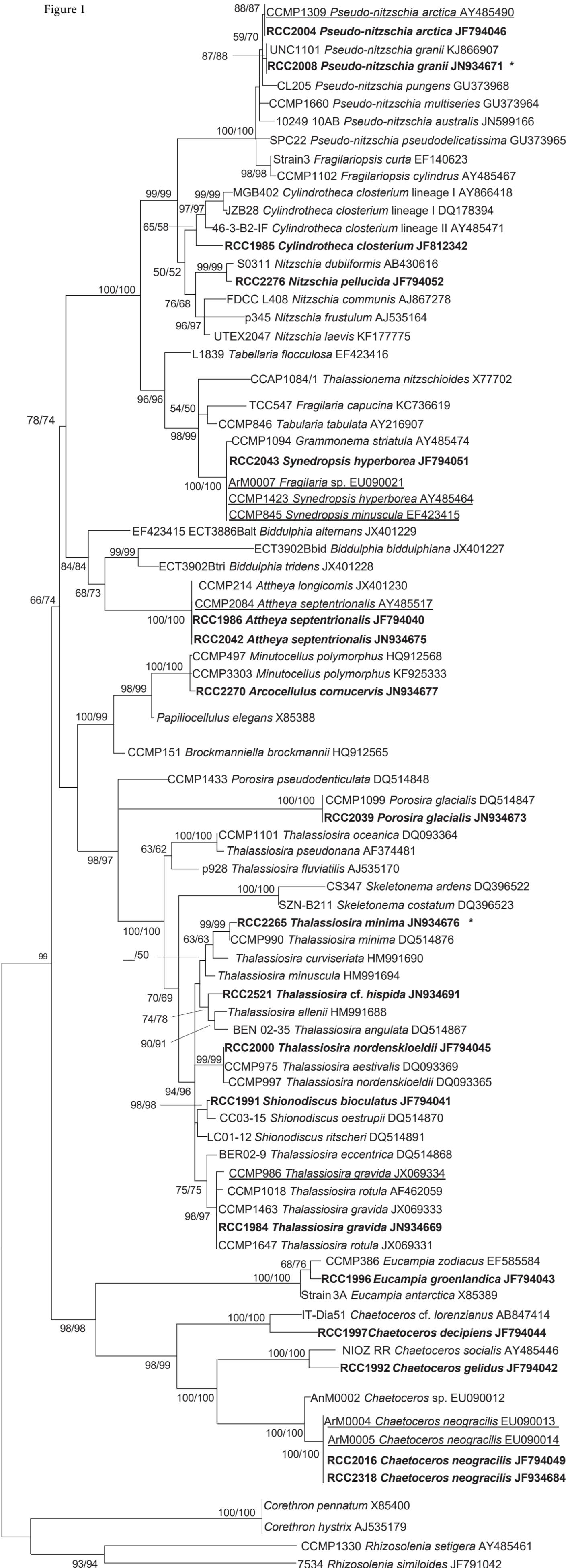
Table S1. Details of the strain isolated during the MALINA cruise and used in the present study.

Most strains are available at Roscoff Culture Collection (RCC)

Table S2. List of the strains and species from which the sequences were used in the present study for the phylogenetic trees. Most strains are currently available at different institutions or culture collections. CCMP: National Centre for Marine Algae and Microbiota (ncma.bigelow.org), UNC: Culture Collection at University of North Carolina (www.unc.edu/), NIOZ: Culture Collection at Netherland Institute for Sea Research (www.nioz.nl), UTEX: Culture Collection of Algae at University of Texas Austin (utex.org/), CCAP: Culture Collection of Algae and Protozoa (www.ccap.ac.uk), TCC: Thonon Culture Collection (www6.inra.fr/cartel-collection_eng), CS: Australia National Algae Culture Collection (www.csiro.au/en/Research/Collections/ANACC/About-our-collection), SZN: Stazione Zoologica Anton Dohrn, Naples (www.szn.it), RCC: Roscoff Culture Collection (<http://roscoff-culture-collection.org>).

Figure S1. Phylogenetic tree of the ITS operon of the *Chaetoceros* sp. strains isolated in the present study. The Antarctic strains of *Chaetoceros* sp. (CCMP187, CCMP189, CCMP190) were used as outgroup. The bootstrap values are indicated next to the branches as for Figure 6.

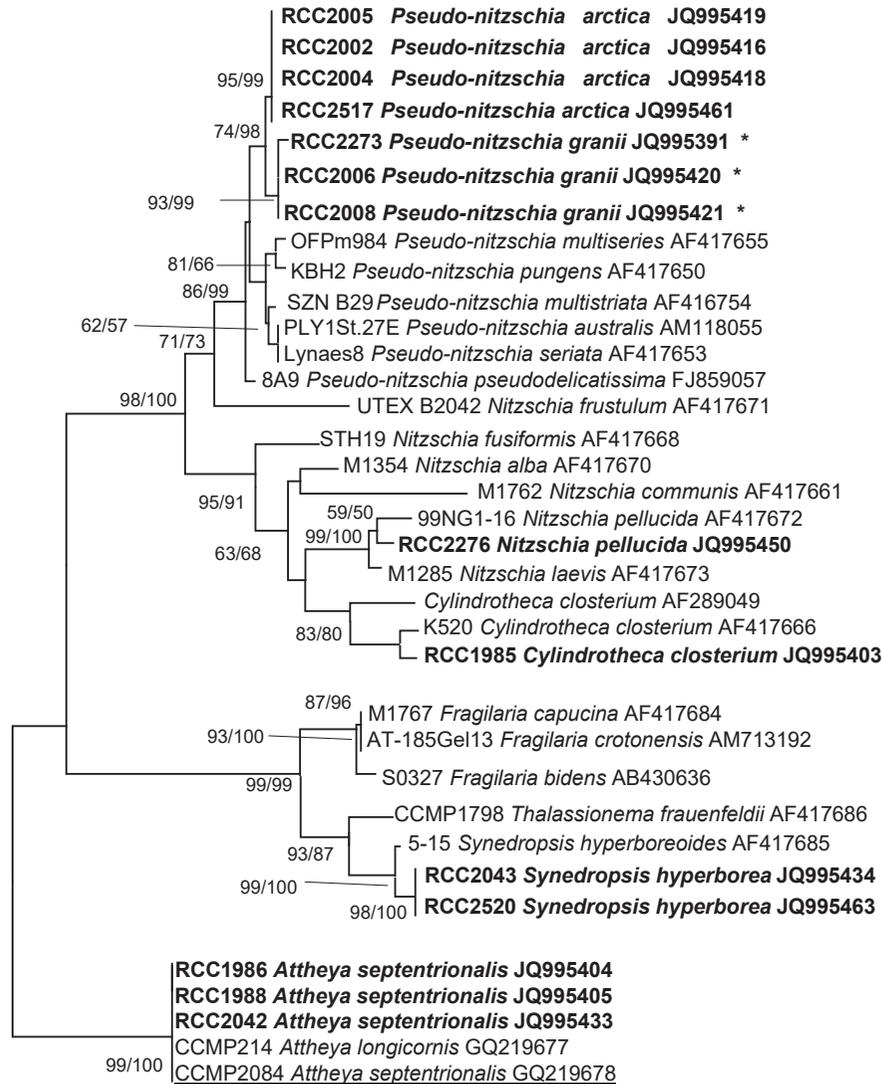
Figure 1



0.02

Figure 2

A



B

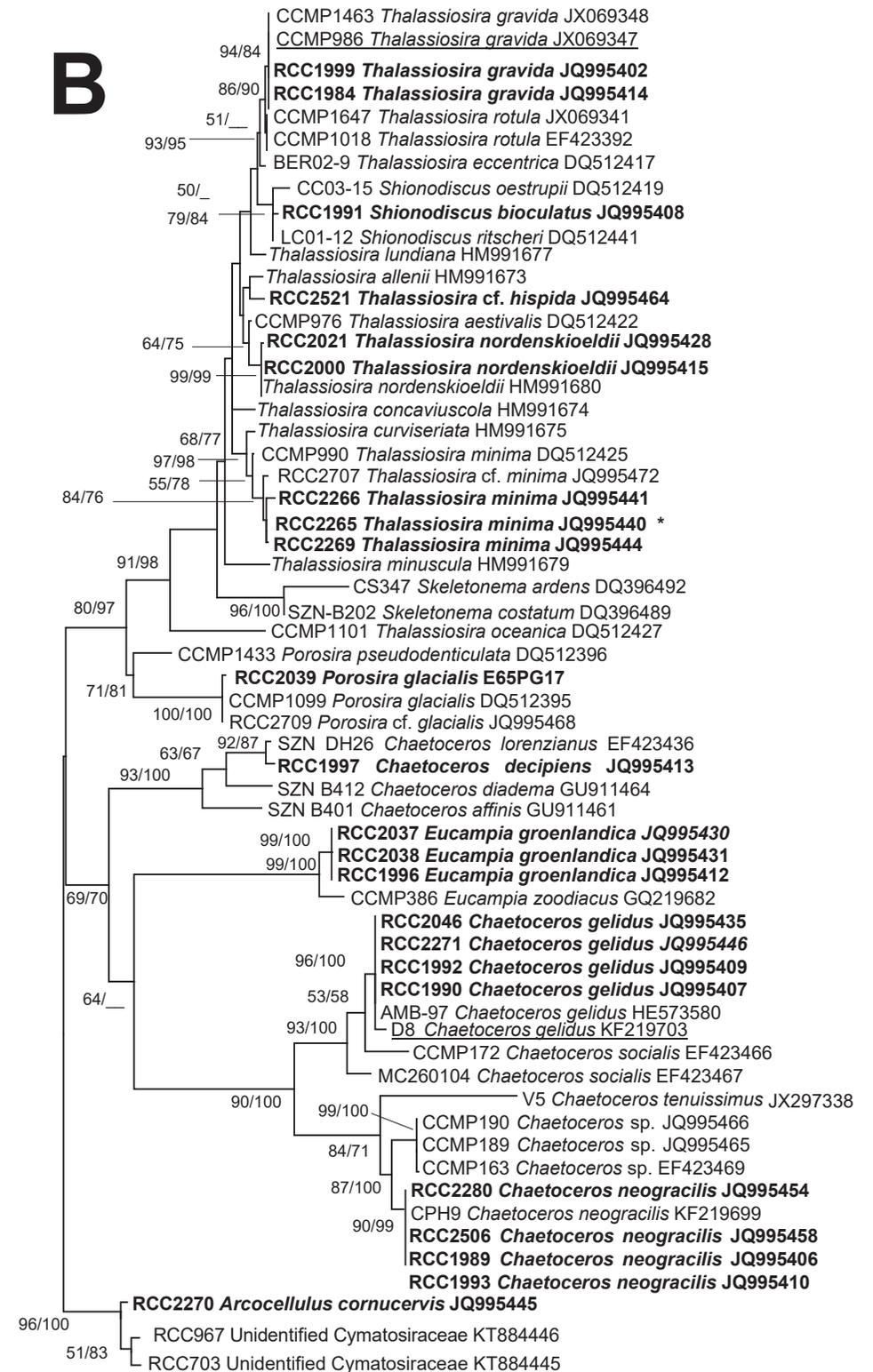
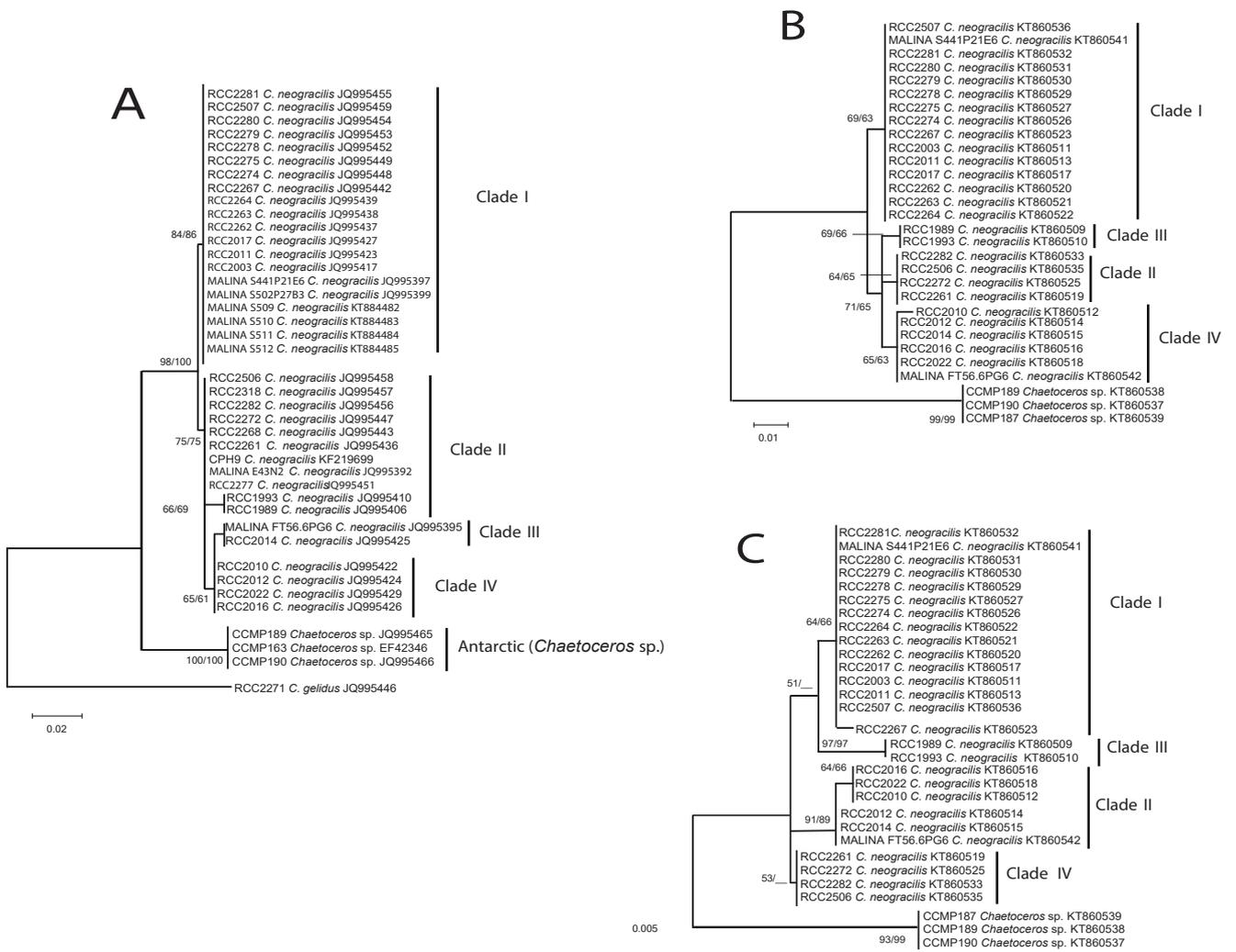
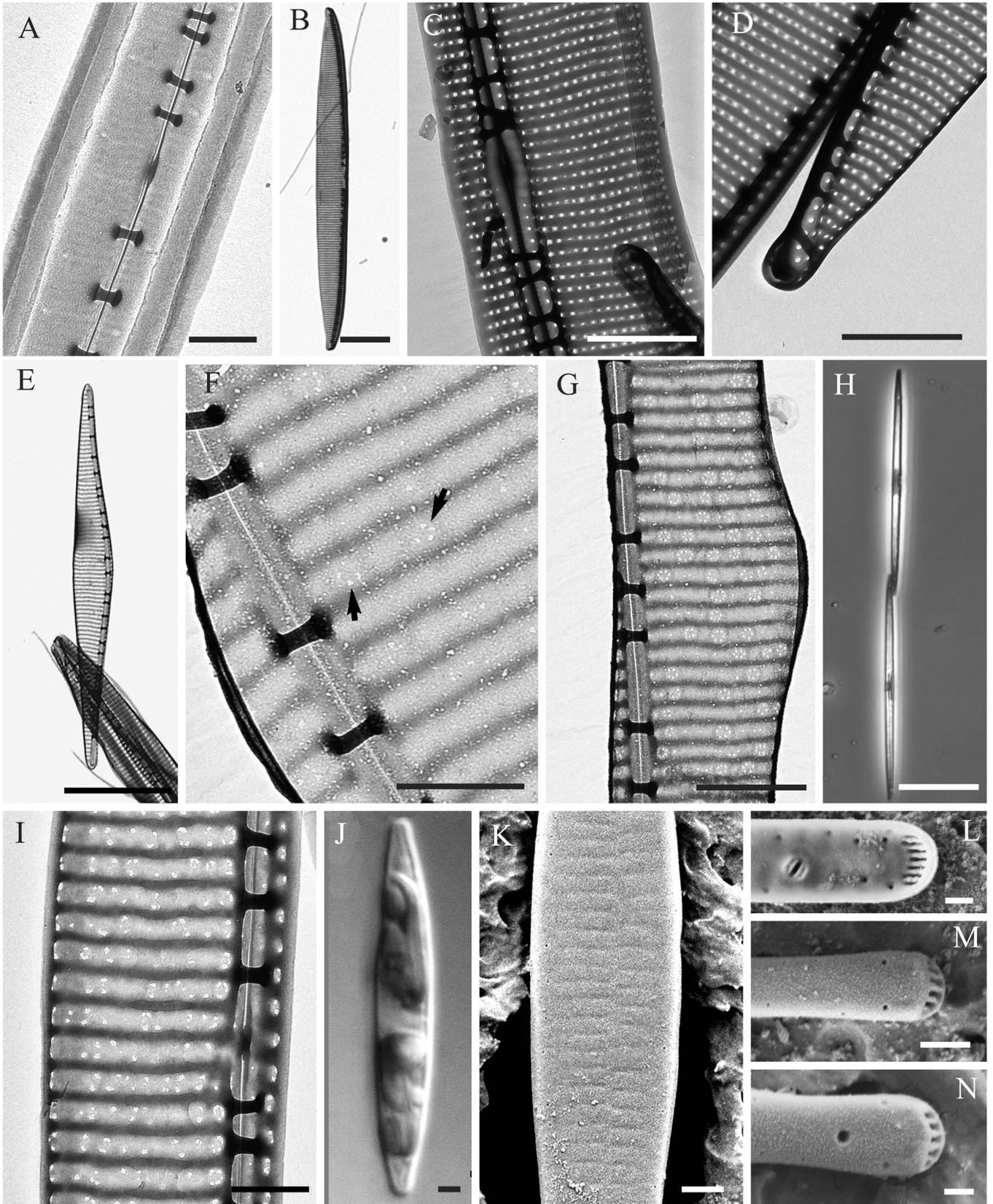
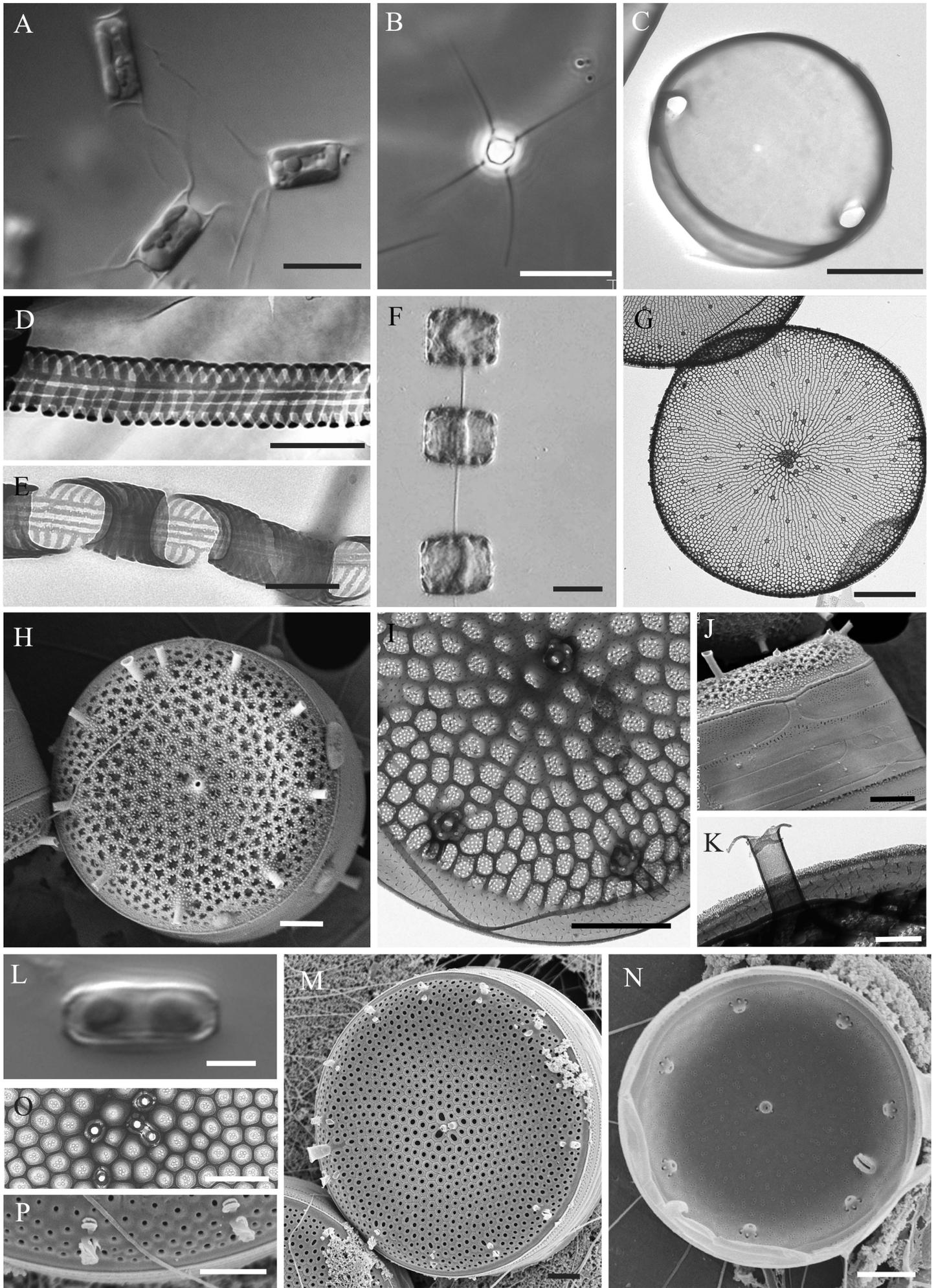
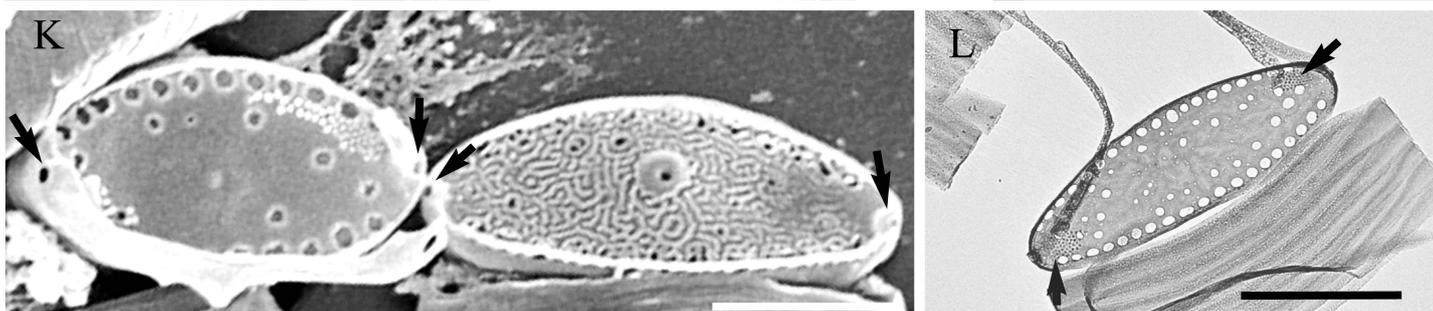
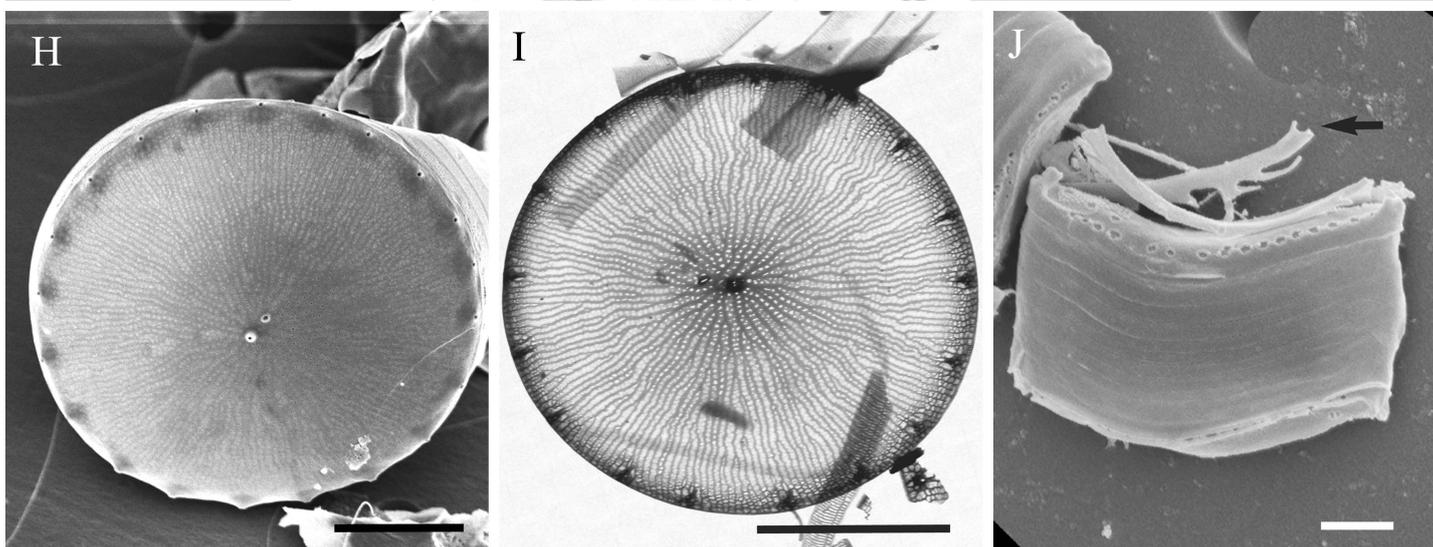
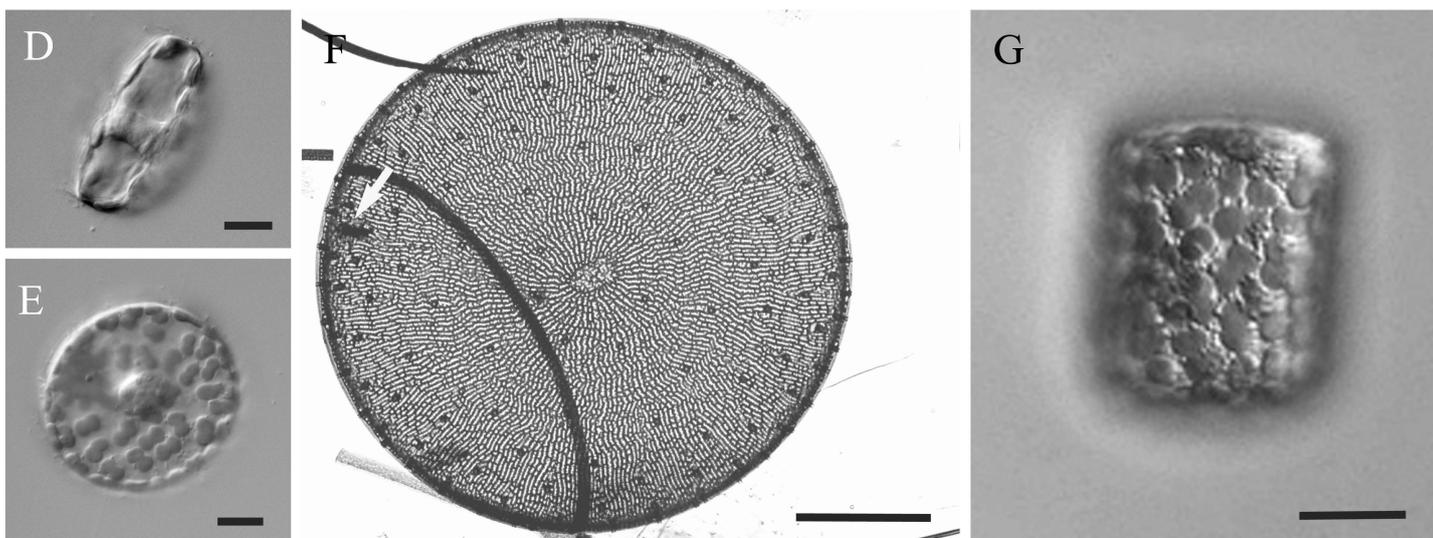
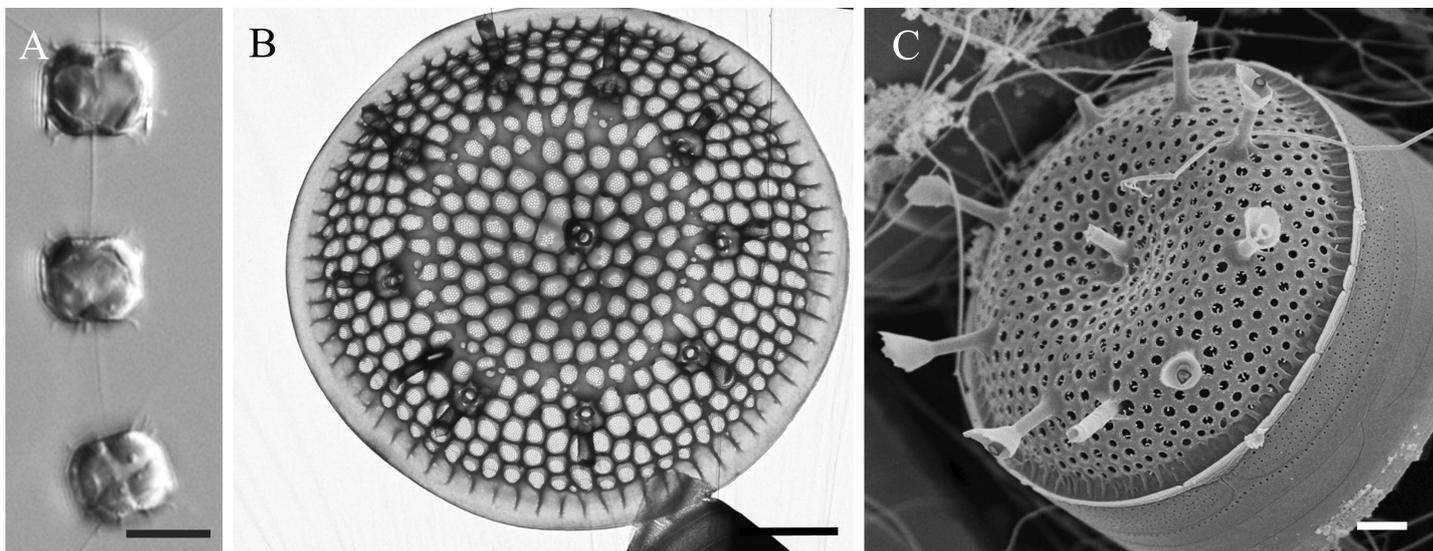


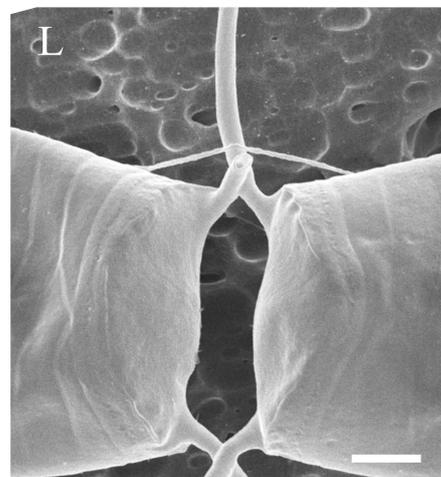
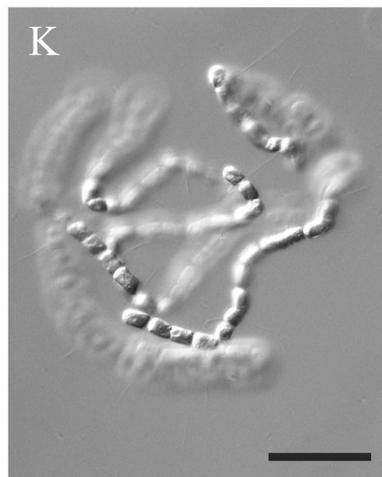
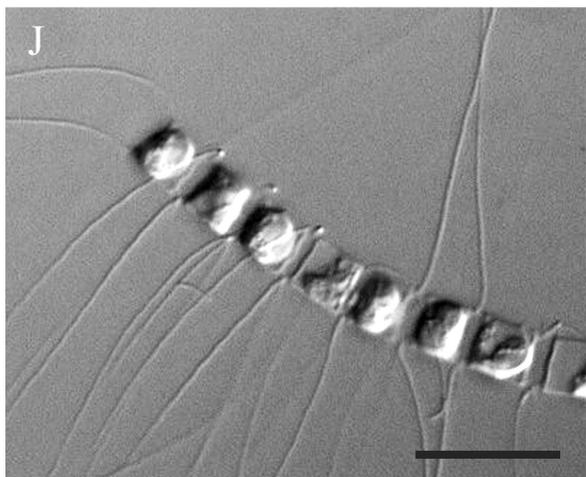
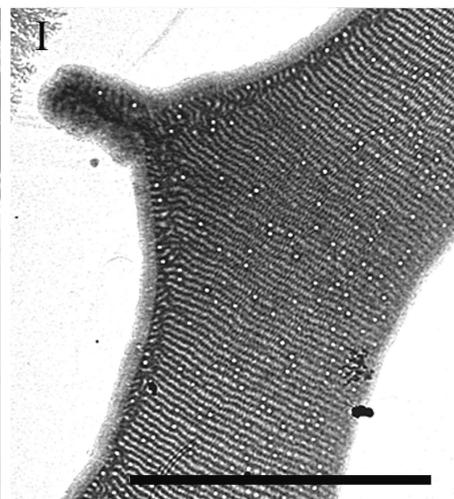
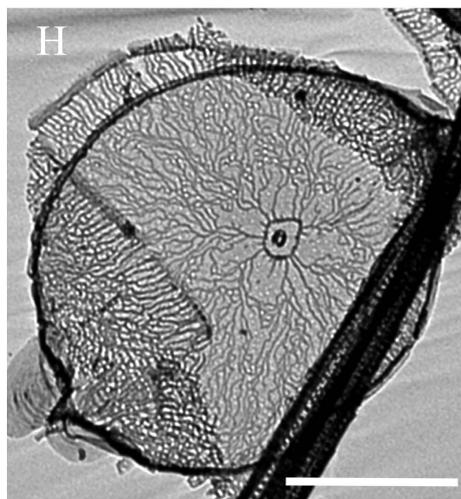
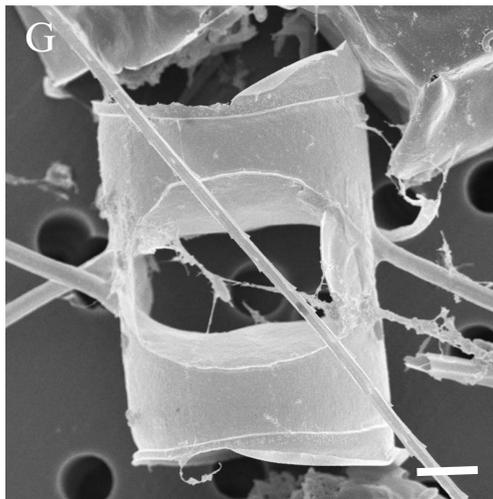
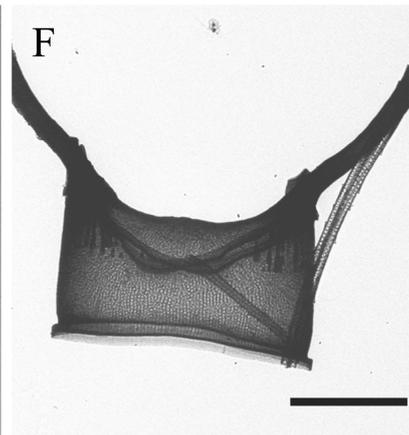
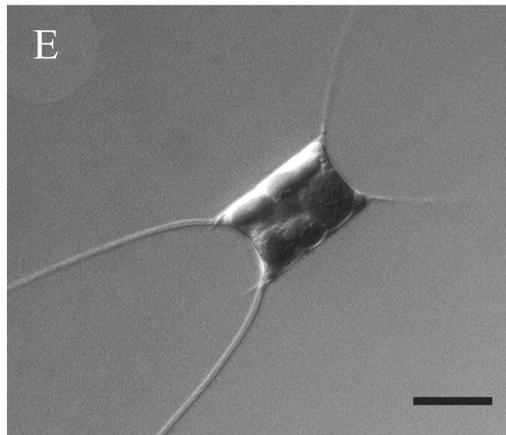
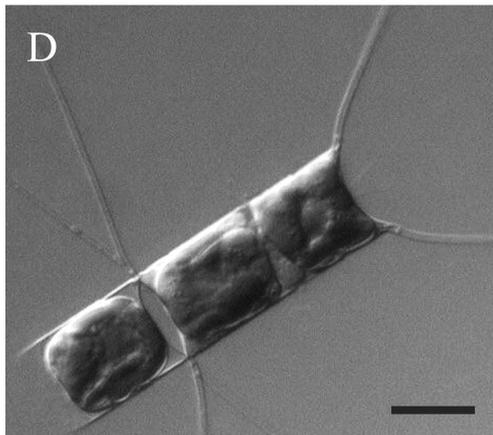
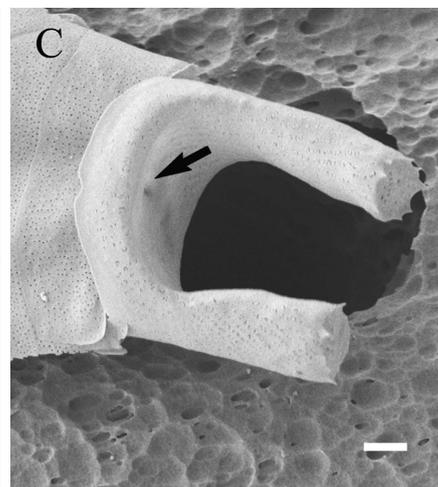
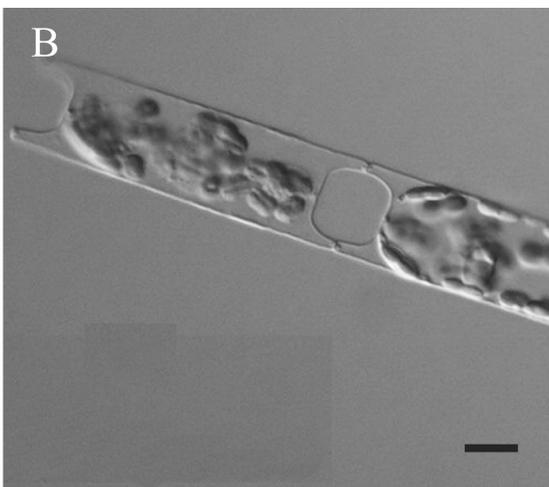
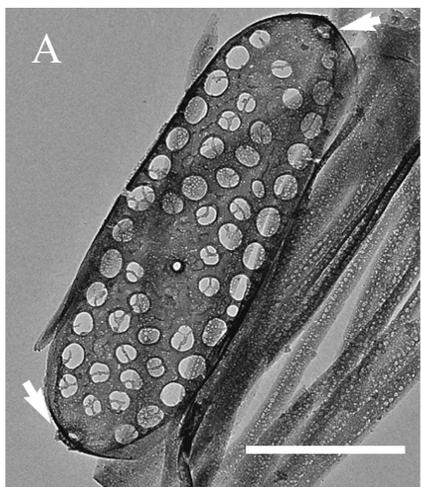
Figure 3











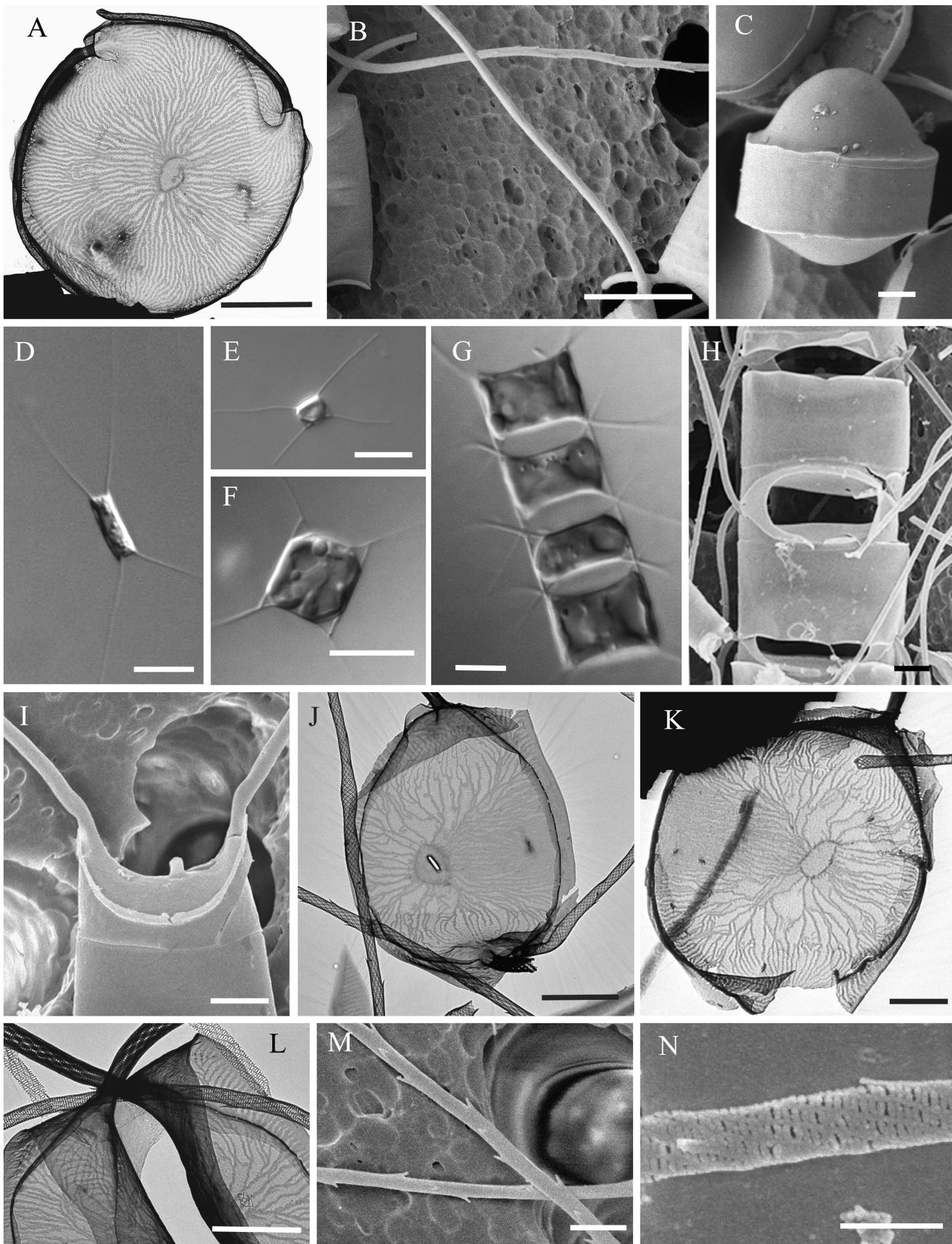
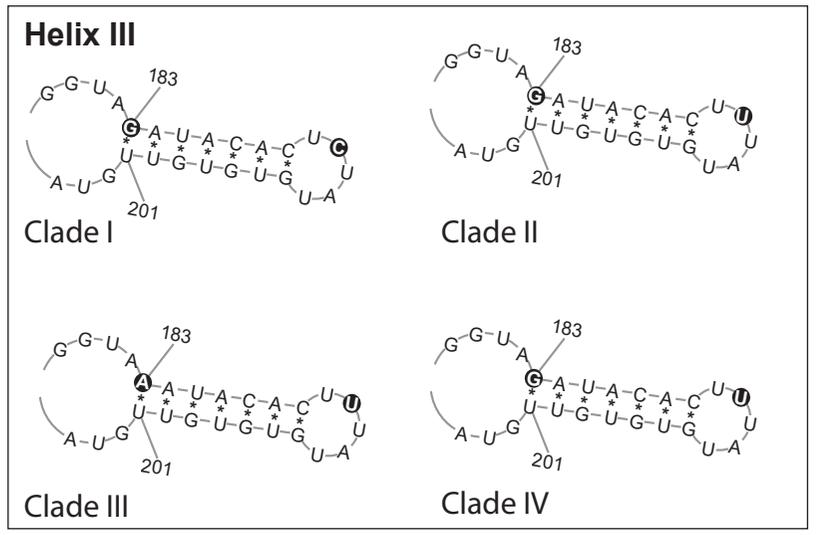
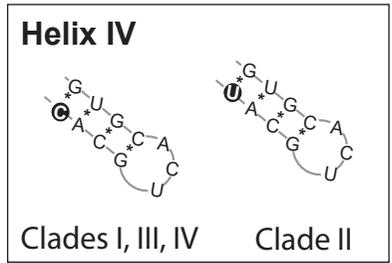
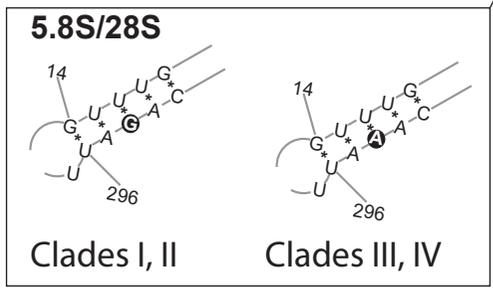
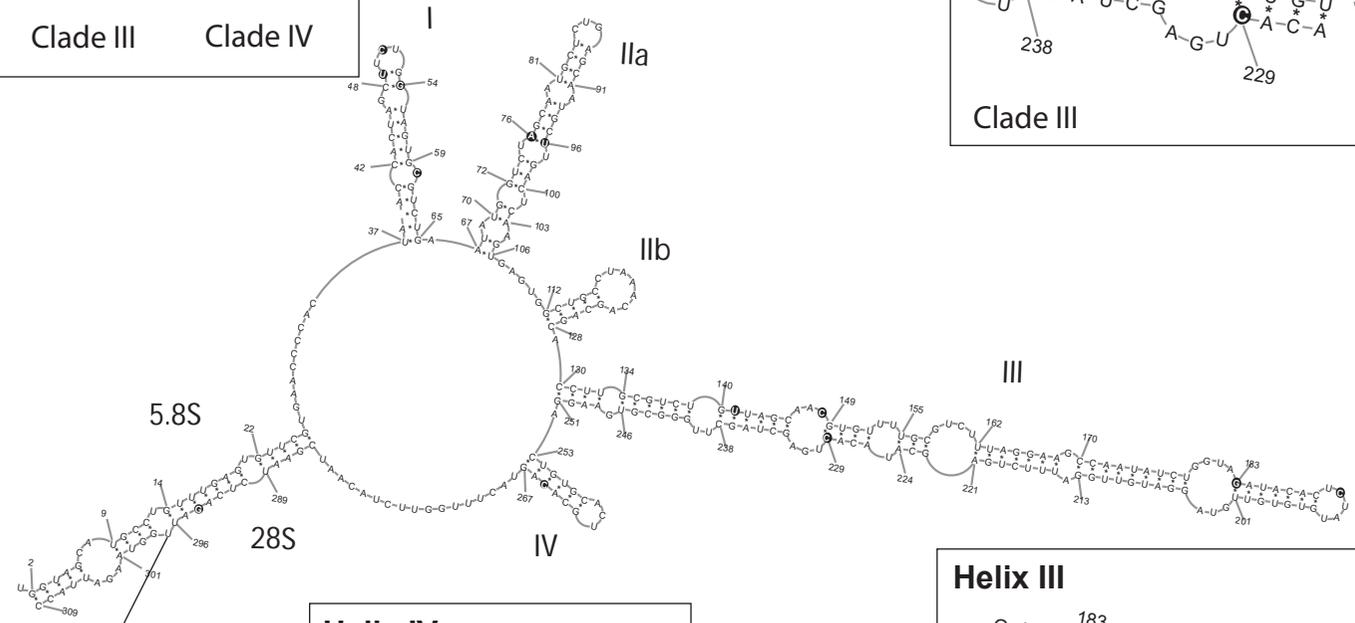
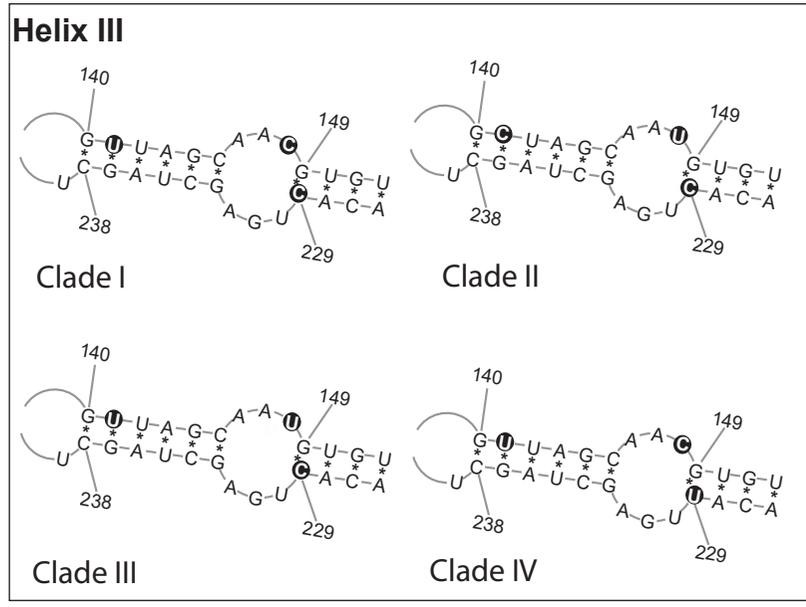
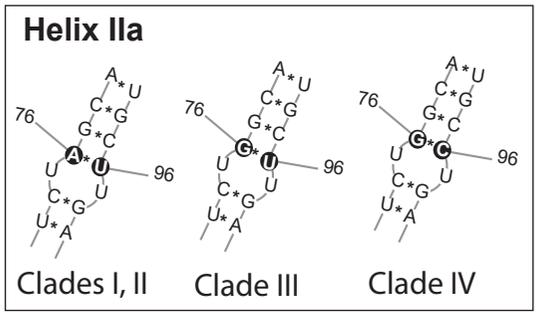
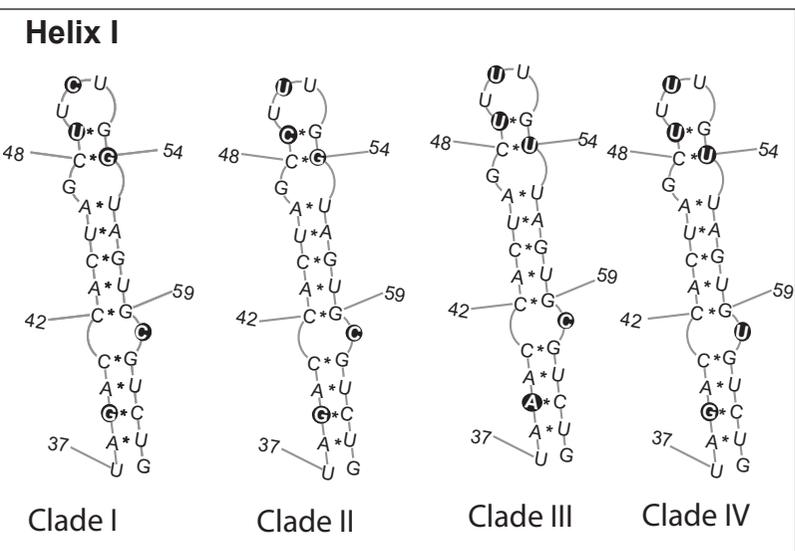
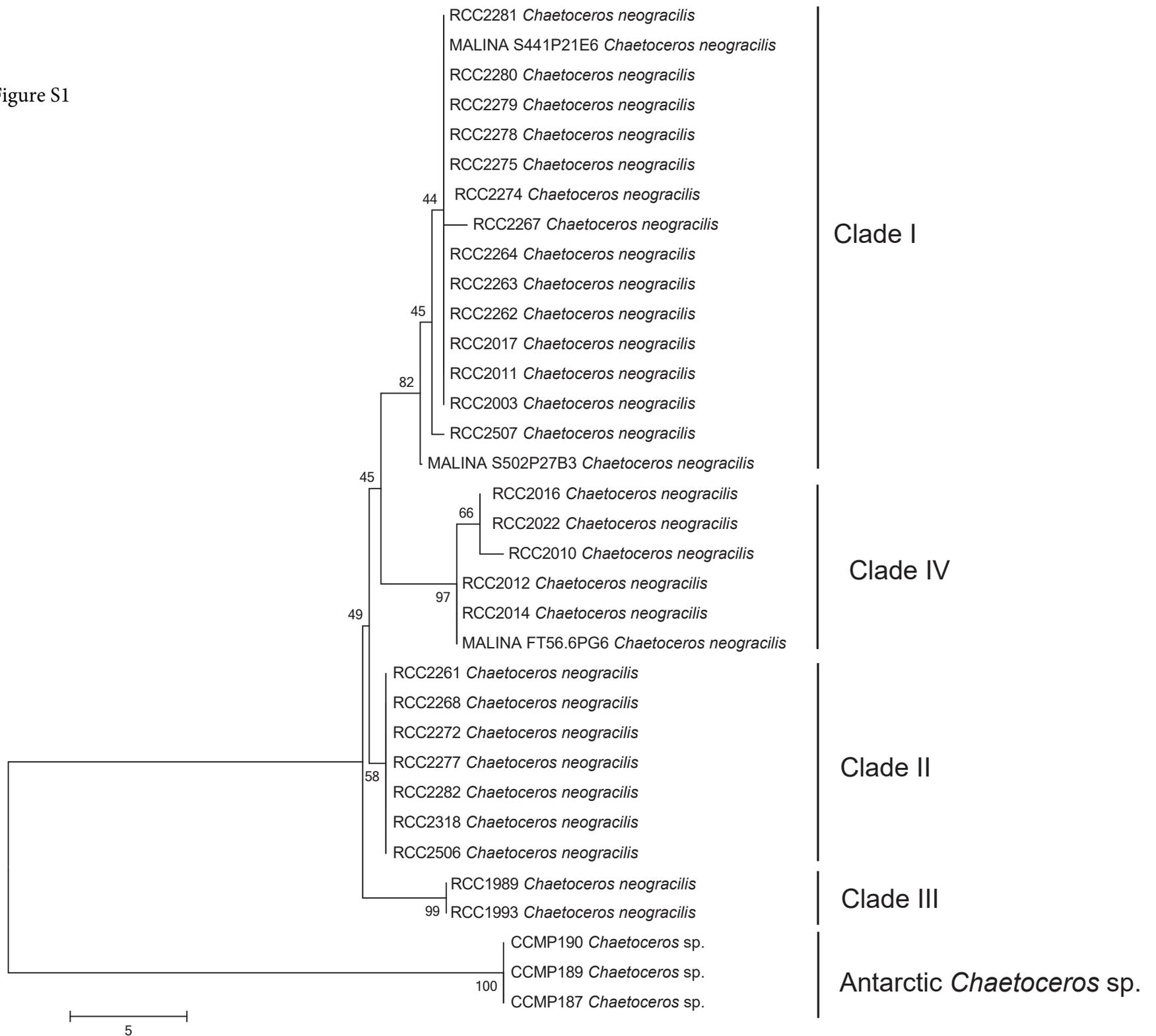


Figure 9



Supplementary Figure S1



Supplementary Table S1

Station	CTD	Depth	Latitude (°N)	Longitude (°W)	Temperature (°C)	Salinity (psu)	Cultures direct FCS ^a	Cultures TFF ^b		Culture Enrichments	
								FCS	Pipette isolation	FCS	Pipette isolation
PAC05		3								1	
PAC06		3	50.06	-139.53	12.1	32.5				1	
PAC08		3	53.36	159.29	11.8	32.7					2
ARC12		3	71.19	159.42	2	30.5	1				
BEA13		3	70.56	145.4	8.8	17.6	2			2	
BEA14		3	70.5	135.5	3.3	25.6	1			1	
110	56	3	71.7	-126.48	4.4	28.7			3		
235	191	3	71.76	-130.83	0	27.3		3			
235	191	25	71.76	-130.83	1.6	29.9		1			
280	42	30	70.87	-130.51	-0.7	32.2			8		
320	82	3	71.57	-133.94	-0.8	27		3			
394	38	3	69.85	-133.49	7	25.1	3				
460	145	3	70.68	-136.05			2				
620	99	3	70.68	-139.63	1.6	22.1		2	3		
620	99	65	70.68	-139.63	-1.1	30.7		1	5		
680	35	3	69.6	-138.23	8.3	14.7			3		
680	35	40	69.6	-138.23	-1.2	31.3		1			
690	31	3	69.49	-137.94	7.4	19	1				
690	31	29	69.49	-137.94	-1.3	31.2					16
760	106	3	70.55	-140.8	0.6	22.3		8	2		
Total							10	19	24	5	18

Supplementary Table S2

Strain ID	Strain name	Authors	Geographical origin	Genbank 18S	Genbank 28S	Genbank ITS
CCMP214	<i>Attheya longicornis</i>	Crawford & Gardner	Gulf of Maine, North Atlantic Ocean	JX401230	GQ219677	
CCMP2084	<i>Attheya septentrionalis</i>	(Østrup) Crawford	Baffin Bay, Arctic	AY485517	GQ219678	
ECT3886Balt	<i>Biddulphia alternans</i>	(Bailey) Van Heurck	Kahana Bay, North Pacific Ocean	JX401229		
ECT3902Bbid	<i>Biddulphia biddulphiana</i>	(J.E.Smith) Boyer	Unknown	JX401227		
ECT3902 Btn	<i>Biddulphia tridens</i>	(Ehrenberg) Ehrenberg	Long Beach, North Pacific Ocean	JX401228		
CCMP151	<i>Brockmanniella brockmanni</i>	(Hustedt) Hasle, Stosch & Syvertsen	Unknown	HQ912565		
SZN B401	<i>Chaetoceros affinis</i>	Lauder	Unknown		GU911461	
SZN-B412	<i>Chaetoceros diadema</i>	(Ehrenberg) Gran	Gulf of Naples, Mediterranean Sea		GU911464	
AMB-97	<i>Chaetoceros gelidus</i>	Chammansinp, Li, Lundholm & Moestrup	Tromsø, North Atlantic Ocean		HE573580	
D8	<i>Chaetoceros gelidus</i>	Chammansinp, Li, Lundholm & Moestrup	Kattegat Bay, North Sea		KF219703	
SZN-DH26	<i>Chaetoceros lorenzianus</i>	Grunow	Gulf of Naples, Mediterranean Sea		EF423436	
IT-Dia51	<i>Chaetoceros cf. lorenzianus</i>	Grunow	Aki nada Sea, Indian Ocean	AB847414		
ArM0004	<i>Chaetoceros neogracilis</i>	(Schütt) VanLandingham	Svalbard, Arctic Ocean	EU090013		
ArM0005	<i>Chaetoceros neogracilis</i>	(Schütt) VanLandingham	Svalbard, Arctic Ocean	EU090014		
CPH9	<i>Chaetoceros neogracilis</i>	(Schütt) VanLandingham	Hellerup Harbour, Baltic Sea		KF219699	
CCMP172	<i>Chaetoceros socialis</i>	Lauder	Friday Harbour, North Pacific Ocean		EF423466	
MC260104	<i>Chaetoceros socialis</i>	Lauder	Gulf of Naples, Mediterranean Sea		EF423467	
NIOZ RR	<i>Chaetoceros socialis</i>	Lauder	Unknown	AY485446		
AnM0002	<i>Chaetoceros sp.</i>		King George Island, Antarctica	EU090012		
CCMP163	<i>Chaetoceros sp.</i>		Southern Ocean		EF426369	
CCMP187	<i>Chaetoceros sp.</i>		Weddell Sea, Antarctica			KT860539
CCMP189	<i>Chaetoceros sp.</i>		Weddell Sea, Antarctica			KT860538
CCMP190	<i>Chaetoceros sp.</i>		Weddell Sea, Antarctica			KT860537
V5	<i>Chaetoceros tenuissimus</i>	Meunier	Black Sea		JX297338	
Unavailable	<i>Corethron pennatum</i>	(Grunow) Ostenfeld	Unknown	X85400		
Unavailable	<i>Corethron hystrix</i>	Hensen	Unknown	AJ535179		
MGB402	<i>Cylindrotheca closterium</i>	(Ehrenberg) Lewin & Reimann	Qindao Bay, Indian Ocean	AY866418		
JZB28	<i>Cylindrotheca closterium</i>	(Ehrenberg) Lewin & Reimann	Qindao Bay, Indian Ocean	DQ178394		
NIOZ (46-3-B2-1F)	<i>Cylindrotheca closterium</i>	(Ehrenberg) Lewin & Reimann	Unknown	AY485471		
K520	<i>Cylindrotheca closterium</i>	(Ehrenberg) Lewin & Reimann	Kattegat Bay, North Sea		AF417666	
Unavailable	<i>Cylindrotheca closterium</i>	(Ehrenberg) Lewin & Reimann	Unknown		AF289049	
Strain 3A	<i>Eucampia antarctica</i>		Unknown	X85389		
CCMP386	<i>Eucampia zodiacus</i>	Ehrenberg	Gulf of Maine, North Atlantic Ocean	EF585584	GQ219682	
S0327	<i>Fragilaria bidens</i>	Heiberg	Unknown		AB430636	
TCC547	<i>Fragilaria capucina</i>	Desmazières	Unknown	KC736619		
M1767	<i>Fragilaria capucina</i>	Desmazières	Cologne pond, Germany		AF417684	
AT-185Gel3	<i>Fragilaria crotonensis</i>	Kitton	Bremen pond, Germany		AM713192	
AnM0007	<i>Fragilaria sp.</i>		Svalbard, Arctic Ocean	EU090021		
Strain 3	<i>Fragilariopsis curta</i>	(Van Heurck) Hustedt	Mertz glacier, Antarctica	EF140623		
CCMP1102	<i>Fragilariopsis cylindrus</i>	(Grunow) Helmcke & Krieger	Islas Orcadas, Antarctica	AY485467		
CCMP1094	<i>Grammonema striatula</i>	C.Agardh	Gulf of Alaska, North Pacific Ocean	AY485474		
CCMP497	<i>Minutocellus polymorphus</i>	(Hargraves & Guillard) Hasle, Stosch. & Syv	Bermuda, North Atlantic Ocean	HQ912568		
CCMP3303	<i>Minutocellus polymorphus</i>	(Hargraves & Guillard) Hasle, Stosch. & Syv	Angola coast, South Atlantic Ocean	KF925333		
M1354	<i>Nitzschia alba</i>	J.C.Lewin & R.A.Lewin	Roscoff, English Channel		AF417670	
M1762	<i>Nitzschia communis</i>	Rabenhorst	Cologne Botanical Garden, Germany		AF417661	
FDCC L408	<i>Nitzschia communis</i>	Rabenhorst	Unknown	AJ867278		
S0311	<i>Nitzschia dubiformis</i>	Hustedt	Unknown	AB430616		
STH19	<i>Nitzschia fusiformis</i>	Grunow	Isefjord, Kattegat Bay, North Sea		AF417668	
p345	<i>Nitzschia frustulum</i>	(Kützing) Grunow	Unknown	AJ535164		
UTEXB2042	<i>Nitzschia frustulum</i>	(Kützing) Grunow	la Jolla, California, North Atlantic Ocean		AF417671	
UTEX2047	<i>Nitzschia laevis</i>	Hustedt	Woods Hole, North Atlantic Ocean	KF177775		
M1285	<i>Nitzschia laevis</i>	Hustedt	Dusseldorf pond, Germany		AF417673	
99NG1-16	<i>Nitzschia pellucida</i>	Grunow	Ishigaki Island, Japan		AF417672	
Unavailable	<i>Papiliocellulus elegans</i>	Hasle, Stosch & Syvertsen	Unknown	X85388		
CCMP1099	<i>Porosira glacialis</i>	(Grunow) Jørgensen	Islas Orcadas, Antarctica	DQ514847	DQ512395	
RCC2709	<i>Porosira cf. glacialis</i>	(Grunow) Jørgensen	Fildes Bay, Antarctica		JQ995468	
CCMP1433	<i>Porosira pseudodenticulata</i>	(Hustedt) Jouse	Ross Sea, Antarctica	DQ514848	DQ512396	
CCMP1309	<i>Pseudo-nitzschia arctica</i>	Percoo & Sarno	Barrow Strait, Canada	AY485490		
10249 10AB	<i>Pseudo-nitzschia australis</i>	Frenguelli	Monterey Bay, North Pacific Ocean	JN599166		
Ply1SL27E	<i>Pseudo-nitzschia australis</i>	Frenguelli	Loch Lihne, North Atlantic Ocean		AM118055	
UNC1101	<i>Pseudo-nitzschia granii</i>	(Hasle) Hasle	Gulf of Alaska, North Pacific Ocean	KJ866907		
CCMP1660	<i>Pseudo-nitzschia multiseries</i>	(Hasle) Hasle	Gulf St Lawrence, North Atlantic Ocean	GU373964		
OFFm984	<i>Pseudo-nitzschia multiseries</i>	(Hasle) Hasle	Ofunato Bay, Indian Ocean		AF417655	
SZNB29	<i>Pseudo-nitzschia multistriata</i>	(Takano) Takano	Gulf of Naples, Mediterranean Sea		AF416754	
SPC22	<i>Pseudo-nitzschia pseudodelicatissima</i>	(Hasle) Hasle	San Pedro Channel, North Pacific Ocean	GU373965		
8A9	<i>Pseudo-nitzschia pseudodelicatissima</i>	(Hasle) Hasle	Thermaikos Gulf, Mediterranean Sea		FJ859057	
CL205	<i>Pseudo-nitzschia pungens</i>	(Grunow ex Cleve) Hasle	Lennoz Channel, North Atlantic Ocean	GU373968		
KBH2	<i>Pseudo-nitzschia pungens</i>	(Grunow ex Cleve) Hasle	Khan Hoa Bay, Indian Ocean		AF417650	
Linaes8	<i>Pseudo-nitzschia seriata</i>	(Cleve) H.Peragallo	Isefjord, Kattegat Bay, North Sea		AF417653	
CCMP1440	<i>Pseudo-nitzschia sp.</i>		McMurdo Sound, Antarctica	GU373969		
CCMP1330	<i>Rhizosolenia setigera</i>	Brightwell	Massachusetts, USA	AY485461		
7534	<i>Rhizosolenia similoides</i>	Cleve	Gulf of Mexico, North Atlantic Ocean	JF791042		
CC03-15	<i>Shionodiscus oestrupii</i>	(Ostenfeld) Alverson, Kang & Theriot	Sargassum Sea, North Atlantic Ocean	DQ514870	DQ512419	
LC01-12	<i>Shionodiscus nitscheri</i>	(Hustedt) Alverson, Kang & Theriot	Drake Passage, Southern Ocean	DQ514891	DQ512441	
CS347	<i>Skeletonema ardens</i>	Sarno & Zingone	Gulf of Carpentaria, Indian Ocean	DQ396522	DQ396492	
SZN-B202	<i>Skeletonema costatum</i>	(Greville) Cleve	Indian River Lagoon, FL, USA		DQ396489	
SZN B211	<i>Skeletonema costatum</i>	(Greville) Cleve	Montevideo coast, South Atlantic Ocean	DQ396523		
CCMP1423	<i>Synedropsis hyperborea</i>	(Grunow) Hasle, Medlin & Syvertsen	Baffin Bay, Arctic	AY485464		
5-15	<i>Synedropsis hyperboreoides</i>	Hasle, Medlin & Syvertsen	Ross Sea, Antarctica		AF417685	
CCMP845	<i>Synedropsis minuscula</i>	(Grunow) Kooistra	Baffin Bay, Arctic	EF423415		
L1839	<i>Tabularia flocculosa</i>	(Roth) Kützing	Unknown	EF423416		
CCMP846	<i>Tabularia tabulata</i>	(Agardh) Snoeijs	Gulf of Alaska, North Pacific Ocean	AY216907		
CCMP1798	<i>Thalassionema frauenfeldii</i>	(Grunow) Tempère & Peragallo	North Atlantic Ocean		AF417686	
CCAP1084/1	<i>Thalassionema nitzschoides</i>	(Grunow) Mereschkowsky	Unknown	X77702		
CCMP975	<i>Thalassiosira aestivalis</i>	Gran	Gulf of Maine, North Atlantic Ocean	DQ093369		
CCMP975	<i>Thalassiosira aestivalis</i>	Gran	Vancouver coast, North Pacific Ocean		DQ512422	
Unknown	<i>Thalassiosira allenii</i>	Takano	Unknown	HM991688	HM991673	
BEN 02-35	<i>Thalassiosira angulata</i>	(Gregory) Hasle	San Joaquin River, California, USA	DQ514867		
Unavailable	<i>Thalassiosira concaviuscula</i>	Makarova	Unknown		HM991674	
Unavailable	<i>Thalassiosira curviseriata</i>	Takano	Unknown	HM991690	HM991675	
BER02-9	<i>Thalassiosira eccentrica</i>	(Ehrenberg) Cleve	San Francisco Bay, North Pacific Ocean	DQ514868	DQ512417	
p928	<i>Thalassiosira fluviatilis</i>	Hustedt	Unknown	AJ535170		
CCMP986	<i>Thalassiosira gravida</i>	Cleve	Tromsø, North Atlantic Ocean	JX069334	JX069347	
CCMP1463	<i>Thalassiosira gravida</i>	Cleve	McMurdo Sound, Antarctica	JX069333	JX069348	
Unknown	<i>Thalassiosira lundiana</i>	Fryxell	Unknown		HM991677	
CCMP990	<i>Thalassiosira minima</i>	Gaarder	Unknown	DQ514876	DQ512425	
RCC2707	<i>Thalassiosira cf. minima</i>	Gaarder	Fildes Bay, Antarctica		JQ995472	
Unavailable	<i>Thalassiosira minuscula</i>	Krasske	Unknown	HM991694	HM991679	
CCMP997	<i>Thalassiosira nordenskiöldii</i>	Cleve	Norwegian Sea, North Atlantic Ocean	DQ093365		
Unavailable	<i>Thalassiosira nordenskiöldii</i>	Cleve	Unknown		HM991680	
CCMP1101	<i>Thalassiosira oceanica</i>	Hasle	Islas Orcadas, Antarctica	DQ093364	DQ512427	
Unavailable	<i>Thalassiosira pseudonana</i>	Hasle & Heimdal	Unknown	AF374481		
CCMP1018	<i>Thalassiosira rotula</i>	Meunier	California Bight, North Pacific Ocean	AF462059	EF423392	
CCMP1647	<i>Thalassiosira rotula</i>	Meunier	Gulf of Naples, Mediterranean Sea	JX069331	JX069341	
RCC967	Unidentified Cymatosiraceae		Chile upwelling, South Pacific Ocean		KT884446	
RCC703	Unidentified Cymatosiraceae		South Africa Coast		KT884445	