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## **Mantoniella beaufortii and Mantoniella baffinensis sp. nov. (Mamiellales, Mamiellophyceae), two new green algal species from the high arctic**

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1 ***MANTONIELLA BEAUFORTII* AND *MANTONIELLA BAFFINENSIS* SP.**  
2 **NOV. (MAMIELLALES, MAMIELLOPHYCEAE),**  
3 **TWO NEW GREEN ALGAL SPECIES FROM THE HIGH ARCTIC<sup>1</sup>**

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33 Running Title: *Mantoniella* species from the high Arctic

34

## 35 Abstract

36 Members of the class Mamiellophyceae comprise species that can dominate picophytoplankton  
37 diversity in polar waters. Yet polar species are often morphologically indistinguishable from  
38 temperate species, although clearly separated by molecular features. Here we examine four  
39 Mamiellophyceae strains from the Canadian Arctic. The 18S rRNA and Internal Transcribed  
40 Spacer 2 (ITS2) gene phylogeny place these strains within the family Mamiellaceae  
41 (Mamiellales, Mamiellophyceae) in two separate clades of the genus *Mantoniella*. ITS2  
42 synapomorphies support their placement as two new species, *Mantoniella beaufortii* and  
43 *Mantoniella baffinensis*. Both species have round green cells with diameter between 3–5  $\mu\text{m}$ , one  
44 long flagellum and a short flagellum ( $\sim 1 \mu\text{m}$ ) and are covered by spiderweb-like scales, making  
45 both species similar to other *Mantoniella* species. Morphologically, *M. beaufortii* and  
46 *M. baffinensis* are most similar to the cosmopolitan *M. squamata* with only minor differences in  
47 scale structure distinguishing them. Screening of global marine metabarcoding datasets indicates  
48 *M. beaufortii* has only been recorded in seawater and sea ice samples from the Arctic while no  
49 environmental barcode matches *M. baffinensis*. Like other Mamiellophyceae genera that have  
50 distinct polar and temperate species, the polar distribution of these new species suggests they are  
51 cold or ice-adapted *Mantoniella* species.

52 *Key index words:* Arctic; ITS; Mamiellophyceae; *Mantoniella*; metabarcoding;  
53 picophytoplankton; polar

54 *Abbreviations:* rRNA, ribosomal RNA; ITS2, internal transcribed spacer 2; compensatory base  
55 change, CBC; hemi-CBC, hCBC; TEM, transmission electron microscopy

56

## 57 Introduction

58 Over the last decades the taxonomy of the green algae has gone through a profound  
59 reorganization. The class Prasinophyceae, initially defined as scaly flagellates (Moestrup and  
60 Throndsen 1988), has been rearranged into several new classes such as the  
61 Chlorodendrophyceae, Chloropicophyceae and Mamiellophyceae (Massjuk 2006, Marin and  
62 Melkonian 2010, Lopes dos Santos et al. 2017b) as well as clades without formal names (Guillou  
63 et al. 2004, Tragin et al. 2016) leading to the class name Prasinophyceae to be abandoned. The  
64 Mamiellophyceae are ecologically successful and particularly dominant in marine coastal waters  
65 (Lopes dos Santos et al. 2017a, Tragin and Vaultot 2018). The first scaled species of  
66 Mamiellophyceae observed were *Mantoniella squamata* (as *Micromonas squamata*, Manton and  
67 Parke 1960) and *Mamiella gilva* (as *Nephroselmis gilva*, Parke and Rayns 1964). Moestrup  
68 (1984) erected the family Mamiellaceae, which included *Mantoniella* and *Mamiella*, with  
69 *Mamiella gilva* designated as the type species. Mamiellophyceae comprises three orders:  
70 Monomastigales, with one freshwater genus *Monomastix*; Dolichomastigales, with two genera  
71 *Crustomastix* and *Dolichomastix*; Mamiellales, which currently comprises five genera  
72 *Bathycoccus*, *Mamiella*, *Mantoniella*, *Micromonas* and *Ostreococcus*. As these genera are  
73 morphologically heterogeneous, with *Micromonas* and *Ostreococcus* lacking scales and  
74 *Bathycoccus* and *Ostreococcus* lacking flagella, the monophyly of Mamiellophyceae was  
75 established based on nuclear and plastid rRNA sequence and secondary structure analyses  
76 (Marin and Melkonian 2010).

77 Molecular analyses of the Mamiellophyceae have permitted the description of otherwise  
78 morphologically indistinguishable cryptic species. For example, wide genetic diversity has been  
79 shown to exist between morphologically identical *Ostreococcus* species where less than 1%

80 difference in the 18S rRNA gene corresponds to up to 30% of variation in orthologous protein  
81 coding sequences (Palenik et al. 2007, Piganeau et al. 2011). From an early stage, 18S rRNA-  
82 defined clades of *Micromonas* and *Ostreococcus* were observed to have distinct geographic  
83 distributions, suggesting their genetic variation reflected adaptations to ecological niches  
84 (Rodríguez et al. 2005, Foulon et al. 2008) and that these clades represented distinct species.  
85 *Ostreococcus* is divided into rare species restricted to estuarine (*O. mediterraneus*) and coastal  
86 environments (*O. tauri*), as well as more abundant oceanic species (*O. lucimarinus* and clade B)  
87 (Demir-Hilton et al. 2011, Treusch et al. 2012, Hu et al. 2016, Simmons et al. 2016).  
88 *Micromonas* cells were observed to be abundant in the Arctic Ocean (Thronsen and Kristiansen  
89 1991, Sherr et al. 2003, Not et al. 2005) that subsequent 18S rRNA analyses revealed them to  
90 belong to a clade with an Arctic distribution (Lovejoy et al. 2007, Balzano et al. 2012).  
91 *Micromonas* has since been revised defining the Arctic clade as the species *M. polaris*, and  
92 species originating from lower latitudes as *M. bravo*, *M. commoda* and *M. pusilla* (Simon et al.  
93 2017). Similarly, in *Mantoniella*, *M. antarctica* was described from the Antarctic whereas  
94 *M. squamata* was cosmopolitan (Marchant et al. 1989).

95 Three picophytoplanktonic strains (RCC2285, RCC2288 and RCC2497) were isolated in the  
96 Canadian Arctic from mesophilic surface water sampled at two sites in the Beaufort Sea in the  
97 summer of 2009 as part of the MALINA cruise (Balzano et al. 2012). A fourth strain (RCC5418)  
98 was subsequently isolated from sea ice collected in Baffin Bay in the spring as part of the Green  
99 Edge project. We performed a combination of molecular, morphological and pigment  
100 characterization of these isolates, which we propose to constitute two novel *Mantoniella* species,  
101 *M. beaufortii* and *M. baffinensis*, restricted to polar environments.

## 102 Methods

103 *Culture conditions.* Strains RCC2285, RCC2288, and RCC2497 were isolated from seawater  
104 collected at two sites (70°30'N, 135°30'W and 70°34'N, 145°24'W) in the Beaufort Sea in the  
105 summer of 2009 as part of the MALINA cruise as described previously (Balzano et al. 2012).  
106 Strain RCC5418 was isolated from the Green Edge project Ice Camp  
107 (<http://www.greenedgeproject.info/>), a sampling site on the sea ice near the village of  
108 Qikiqtarjuaq (67°28.784N, 63°47.372W). Sampling was conducted between 20 April and 27  
109 July, 2016, beginning in completely snow covered conditions followed by bare ice and ending  
110 when the ice was broken out. Sea ice from 23 May 2016 was melted overnight and 200 mL was  
111 gravity filtered (Sartorius filtration system) through 3 µm pore size polycarbonate filters  
112 (Millipore Isopore membrane, 47 mm). 500 µL of filtrate was enriched by addition to 15 mL of  
113 L1 medium (NCMA, Bigelow Laboratory for Ocean Sciences, USA). The enrichment culture  
114 was purified by dilution to 10 cells per well in a 96 deep-well plate (Eppendorf) and incubated  
115 under white light (100 µE m<sup>-2</sup> s<sup>-1</sup>) in a 12:12 h light: dark cycle at 4°C. Cell growth was observed  
116 by the development of coloration after a few weeks. Culture purity was assessed by flow  
117 cytometry (Becton Dickinson, Accuri C6). After confirmation of the purity, the culture was  
118 transferred in a 50 mL ventilated flask (Sarstedt). Cultures are maintained in the Roscoff Culture  
119 Collection (<http://roscoff-culture-collection.org/>) in K/2 (Keller et al. 1987) or L1 medium at  
120 4°C under a 12:12 h light: dark cycle at 100 µE light intensity. RCC2285 has been lost from  
121 culture since molecular analyses (described below) were performed. For pigment analysis and  
122 electron microscopy, RCC2288 was grown at 7°C under continuous light at 100 µE intensity in  
123 L1 medium prepared using autoclaved seawater from offshore Mediterranean Sea water diluted  
124 10% with MilliQ water and filtered prior to use through 0.22 µm filters. Holotype specimens

125 were deposited in O (Natural History Museum, University of Oslo), herbarium acronym follows  
126 Thiers (2019).

127

128 *Sequences.* Nuclear 18S rRNA and the Internal Transcribed Spacers (ITS) 1 and 2, as well as the  
129 5.8S rRNA gene were retrieved from GenBank for strains RCC2288, RCC2497 and RCC2285  
130 (Balzano et al. 2012). For RCC5418 and RCC5150 (*M. antarctica*), cells were harvested in  
131 exponential growth phase and concentrated by centrifugation. Total nucleic acids were extracted  
132 using the Nucleospin Plant II kit (Macherey-Nagel, Düren, DE) following the manufacturer's  
133 instructions. The nearly full length nuclear 18S rRNA gene (only RCC5418) and the region  
134 containing the Internal Transcribed Spacers (ITS) 1 and 2, as well as the 5.8S rRNA gene were  
135 obtained by PCR amplification using universal primers (Supplementary Table 1). PCR products  
136 were directly sequenced at the Macrogen Company (Korea) and sequences have been deposited  
137 to Genbank under accession numbers MH516003, MH516002 and MH542162.

138

139 *ITS2 secondary structure.* The ITS2 secondary structure from the strains listed in Table 1 was  
140 predicted using the Mfold web interface (Zuker 2003) under the default options with the folding  
141 temperature fixed at 37°C, resulting in multiple alternative folding patterns per sequence. The  
142 preliminary structure for each sequence was chosen based on similarities found among the other  
143 structures proposed for Mamiellophyceae (Marin and Melkonian 2010, Simon et al. 2017) as  
144 well as on the presence of previously defined ITS2 hallmarks defined by Coleman (Mai and  
145 Coleman 1997, Coleman 2000, 2003, 2007). Exported secondary structures in Vienna format and  
146 the respective nucleotide sequences were aligned, visualized using 4SALE version 1.7 (Seibel et  
147 al. 2008) and manually edited through extensive comparative analysis of each position



148 (nucleotide) in sequences from representatives of the Mamiellophyceae. The ITS2  
149 synapomorphy analysis was confined to those positions that formed conserved base pairs in all  
150 members of the Mamiellaceae order and the resulting intramolecular folding pattern (secondary  
151 structure) of *Mantoniella* was drawn using CorelDRAW X7. A Vienna file containing the ITS2  
152 sequences and secondary structure is available at  
153 <https://doi.org/10.6084/m9.figshare.7472153.v1>.

154  
155 *Phylogenetic analyses.* Nuclear 18S rRNA sequences belonging to members of  
156 Mamiellophyceae were retrieved from GenBank (<http://www.ncbi.nlm.nih.gov/>). Two  
157 environmental sequences (similar to strain sequences) were included in addition to the sequences  
158 obtained from the cultures. Sequences were also obtained for the ITS2 region located between  
159 the 5S and 23S rRNA genes. However, no environmental sequences were available to be  
160 included in the 18S/ITS phylogenetic analyses.

161 Twenty-seven nuclear 18S rRNA and fourteen ITS2 sequences were aligned with MAFFT  
162 using the E-INS-i and G-INS-i algorithms respectively (Kato and Toh 2008). Alignments were  
163 visualized and manually edited using Geneious 10.2.5 (Kearse et al. 2012). The ITS2 alignment  
164 was further edited on the basis of conserved secondary structures (see above). The nuclear 18S  
165 rRNA and ITS2 sequences from the Mamiellaceae members were concatenated using Geneious  
166 10.2.5 (Kearse et al. 2012). Lengths of the resulting alignments were 1567 bp for 18S rRNA  
167 (1242 identical sites, 295 variable and 191 parsimony-informative sites) and 1875 bp for  
168 concatenated 18S-ITS sequences (1544 identical sites, 302 variable and 179 parsimony-  
169 informative).

170 Phylogenetic reconstructions with two different methods, maximum likelihood (ML) and  
171 Bayesian analyses, were performed using the nuclear Mamiellophyceae 18S rRNA and  
172 Mamiellaceae concatenated 18S/ITS2 alignments.

173 The K2 + G + I model was selected for both sequence datasets based on the substitution  
174 model selected through the Akaike information criterion (AIC) and the Bayesian information  
175 criterion (BIC) options implemented in MEGA 6.06 (Tamura et al. 2013). ML analysis was  
176 performed using PhyML 3.0 (Guindon et al. 2010) with SPR (Subtree Pruning and Regrafting)  
177 tree topology search operations and approximate likelihood ratio test with Shimodaira-  
178 Hasegawa-like procedure. Markov chain Monte Carlo iterations were conducted for 1,000,000  
179 generations sampling every 100 generations with burning length 100,000 using MrBayes 3.2.2  
180 (Ronquist and Huelsenbeck 2003) as implemented in Geneious (Kearse et al. 2012). Nodes were  
181 considered as well supported when SH-like support values and Bayesian posterior probabilities  
182 were higher than 0.8 and 0.95 respectively. The same criteria were used to represent the  
183 sequences on the phylogenetic trees. Alignments are available at  
184 <https://doi.org/10.6084/m9.figshare.7472153.v1>.

185 *Screening of environmental 18S rRNA sequencing datasets.* High-throughput sequencing  
186 metabarcodes (V4 and V9 hypervariable regions) were obtained from several published polar  
187 studies, as well as from the global sampling efforts Tara Oceans and Ocean Sampling Day  
188 (OSD) (see Supplementary Table 2 for the full details and references for each project). We  
189 screened these data as well as GenBank by BLASTn (98% identity cut-off) using RCC2288 18S  
190 rRNA gene sequence as the search query. We aligned the retrieved environmental sequences and  
191 metabarcodes with that of RCC2285, RCC2288, RCC2497, and RCC5418 using MAFFT as  
192 implemented in Geneious version 10.0.7 (Kearse et al. 2012). This allowed the determination of

193 sequence signatures diagnostic of this species for both V4 and V9 (Supplementary Figures 1 and  
194 2). The oceanic distribution of stations where cultures, clones and metabarcodes having these  
195 signatures, as well as the stations from the metabarcoding surveys where no matching  
196 metabarcodes have been found, were plotted with the R libraries ggplot2 and rworldmap. The R  
197 script is available at <https://vaulot.github.io/papers/RCC2288.html>.

198

199 *Light microscopy.* Cells were observed using an Olympus BX51 microscope (Olympus,  
200 Hamburg, Germany) with a 100× objective using differential interference contrast (DIC) and  
201 imaged with a SPOT RT-slider digital camera (Diagnostics Instruments, Sterling Heights, MI,  
202 USA).

203 For video-microscopy, cultures from RCC2288 and RCC2497 were observed with an inverted  
204 Olympus IX70 inverted microscope using an x40 objective and equipped with an Infinity X  
205 camera (<https://www.lumenera.com/products/microscopy/infinityx-32.html>). Short sequences  
206 were recorded and edited with the Video de Luxe software ([http://www.magix.com/fr/video-](http://www.magix.com/fr/video-deluxe/)  
207 [deluxe/](http://www.magix.com/fr/video-deluxe/)). Films were uploaded to Youtube ([https://www.youtube.com/channel/UCsYoz-](https://www.youtube.com/channel/UCsYoz-aSJIJesyDNj6ZVolQ/videos)  
208 [aSJIJesyDNj6ZVolQ/videos](https://www.youtube.com/channel/UCsYoz-aSJIJesyDNj6ZVolQ/videos)). Video microscopy of swimming behavior of RCC2288  
209 (<https://youtu.be/CGKNxzfGUvQ>), RCC2497 (<https://youtu.be/rRNuk5Lx7Aw>), and RCC5418  
210 (<https://youtu.be/xoxCE11cv4Q>). The recording protocol is available at  
211 [dx.doi.org/10.17504/protocols.io.k24cygw](https://dx.doi.org/10.17504/protocols.io.k24cygw).

212

213 *Transmission Electron Microscopy.* Positive-stained whole mount cells were prepared as  
214 described by Moestrup (1984), where cultures were directly deposited on formvar coated copper  
215 grids and stained with 2% uranyl acetate. TEM thin-sections was performed as previously

216 described (Derelle et al. 2008). Briefly, fixed RCC2288 cells (1% glutaraldehyde) from an  
217 exponentially growing culture were suspended in molten (37°C) 1% low melting point agarose.  
218 The agarose cell plug was fixed, washed, dehydrated in ethanol and embedded in Epon 812.  
219 Ultra-thin sections (80–90 nm) were placed on a 300 mesh copper grid and stained with uranyl  
220 acetate for 15 min, followed by lead citrate staining for 2 min. The cells were visualized with  
221 Hitachi H 7500 and H-9500 transmission electron microscopes.

222  
223 *Pigment analysis.* Pigments were extracted from RCC2288 cells in late exponential phase as  
224 previously described (Ras et al. 2008). Briefly, cells were collected on 0.7 µm particle retention  
225 size filters (GF/F Whatman), pigments extracted for 2 hours in 100% methanol, then subjected to  
226 ultrasonic disruption and clarified by filtration through 0.2 µm pore-size filters (PTFE). Pigments  
227 were detected using high performance liquid chromatography (HPLC, Agilent Technologies  
228 1200) over the 24 h after the extraction.

## 229 Results and Discussion

230 *Taxonomy section. Mantoniella beaufortii* Yau, Lopes dos Santos and Eikrem sp. nov.

231 Description: Cells round measuring  $3.7 \pm 0.4$  µm in diameter with one long ( $16.3 \pm 2.6$  µm)  
232 and one short flagellum (~1 µm). Cell body and flagella covered in imbricated spiderweb scales.  
233 Flagellar hair scales present composed of two parallel rows of subunits. Long flagellum tip has  
234 tuft of three hair scales. Scales produced in Golgi body. Golgi body located beneath and to one  
235 side of basal bodies. One green chloroplast with pyrenoid surrounded by starch and a stigma  
236 composed of a single layer of oil droplets (~0.1 µm). Ejectosomes composed of fibrils located at  
237 periphery of cell. Cell bodies with sub-quadrangular to oval scales (~0.2 µm). Body scales

238 heptaradial, with seven major spokes radiating from center, number of spokes increasing towards  
239 the periphery. Six or more concentric ribs divide the scale into segments. Flagella with  
240 hexaradial oval scales composed of six spokes increasing in number towards the periphery. Six  
241 or more concentric ribs divide the scale into segments. Combined nucleotide sequences of the  
242 18S rRNA (JN934679) and ITS2 rRNA (JQ413369) are species specific.

243 Holotype: Accession number O-A10010, plastic embedded specimen, 14 July 2009, from  
244 surface water, MALINA cruise leg 1b. Figure 4 shows cells from the embedding. Culture  
245 deposited in The Roscoff Culture Collection as RCC2288.

246 Type locality: Beaufort Sea in the Arctic Ocean (70°30'N, 135°30'W).

247 Etymology: Named for its geographical provenance.

248

249 *Mantoniella baffinensis* Yau, Lopes dos Santos and Eikrem sp. nov.

250 Description: Cells measuring  $4.7 \pm 0.5 \mu\text{m}$  with one long flagellum of  $21.8 \pm 5.1 \mu\text{m}$  and one  
251 short flagellum ( $\sim 1 \mu\text{m}$ ). Cell body and flagella covered in imbricated spiderweb scales. Flagellar  
252 hair scales present composed of two parallel rows of subunits. Long flagellum tip has tuft of  
253 three hair scales. Cell bodies with sub-quadrangular to oval scales ( $\sim 0.2 \mu\text{m}$ ). Body scales  
254 octaradial with eight major radial spokes radiating from center, number of spokes increasing  
255 towards the periphery. Seven or more concentric ribs divide the scale into segments. Flagella  
256 with heptaradial, oval scales composed of seven spokes increasing in number towards the  
257 periphery. Six or more concentric ribs divide the scale into segments. Combined nucleotide  
258 sequences of the nuclear 18S rRNA (MH516003) and ITS2 rRNA (MH542162) are species  
259 specific.

260 Holotype: Accession number O-A10011, plastic embedded specimen, 23 May 2016, from  
261 surface sea ice, Green Edge project Ice Camp. Culture deposited in The Roscoff Culture  
262 Collection as RCC5418.

263 Type locality: Surface sea ice off the coast of Baffin Island in Baffin Bay (67°28'N, 63°46'W).

264 Etymology: Named for its geographical provenance.

265

266 *Phylogeny and ITS signatures.* The phylogenetic tree based on nearly full-length nuclear 18S  
267 rRNA sequences obtained from the novel polar strains RCC2288, RCC2285, RCC2497 and  
268 RCC5418 (Table 1), and environmental sequences retrieved from GenBank indicated that these  
269 strains belong to the family Mamiellaceae (Supplementary Figure 3). The analysis also recovered  
270 the major genera within Mamiellales: *Bathycoccus*, *Ostreococcus*, *Micromonas*, *Mantoniella* and  
271 *Mamiella* (Marin and Melkonian 2010). Dolichomastigales and Monomastigales were the basal  
272 orders in Mamiellophyceae with *Monomastix opisthostigma* type species used as an outgroup.  
273 Strains RCC2485, RCC2288 and RCC2497 isolated during the MALINA cruise in the Beaufort  
274 Sea and strain RCC5418 isolated from Baffin Bay during the Green Edge project Ice Camp  
275 formed a well-supported clade together with two environmental sequences (clone MALINA  
276 St320 3m Nano ES069 D8 and clone 4-E5), which also originated from Arctic Ocean samples.  
277 The two described *Mantoniella* species (*M. squamata* and *M. antarctica*) were not monophyletic  
278 in our analysis using the nuclear 18S rRNA, as reported by Marin and Melkonian (2010)  
279 (Supplementary Figure 3).

280 In contrast, the phylogenetic tree based on concatenated 18S/ITS2 alignments suggested that  
281 our strains belong in *Mantoniella* (Figure 1). The grouping of our strains within *Mantionella* in  
282 the concatenated 18S/ITS tree was consistent with a recent nuclear multigene phylogeny based

283 on 127 concatenated genes from related Chlorophyta species that also included RCC2288 with  
284 *Mantoniella* species (Lopes dos Santos et al. 2017b). This indicated the 18S/ITS2 tree reflects  
285 the evolutionary history of the nuclear genome supporting the position of *Mantoniella* and our  
286 strains diverging from the same common ancestor.

287 The average distance between strains RCC2485, RCC2288 and RCC2497 was low (0.5% of  
288 segregating sites over the near full-length 18S rRNA gene), suggesting that these strains  
289 corresponded to a single species that we named *Mantoniella beaufortii* (see Taxonomy section).  
290 In contrast, the well-supported placement of strain RCC5418 on an earlier diverging branch  
291 within the *Mantoniella* clade, as well as the 1% average distance between RCC5418 and the  
292 other strains, suggested it represents another species, named here *Mantoniella baffinensis*.

293 To substantiate the description of *M. beaufortii* and *M. baffinensis* as new species, we  
294 investigated ITS2 synapomorphies of the different *Mantoniella* species. Although the use of  
295 ITS2 in taxonomy should be considered with caution (Müller et al. 2007, Caisová et al. 2011),  
296 several studies have shown the power of using ITS2 sequences in delimiting biological species,  
297 especially in microalgal studies (e.g. Coleman 2007, Caisová et al. 2011) including green algae  
298 (Subirana et al. 2013, Simon et al. 2017). For example, ITS sequencing contributed to  
299 distinguishing the Arctic diatom *Chaetoceros neogracilis* from an Antarctic *Chaetoceros* sp. that  
300 shared nearly identical 18S rRNA genes (Balzano et al. 2017). The analysis of ITS2 secondary  
301 structure in addition to molecular signatures of nuclear and plastid SSU rRNA genes supported  
302 the description of Chloropicophyceae clades as distinct species, despite the absence of clear  
303 morphological differences (Lopes dos Santos et al. 2017b). This conclusion has been further  
304 supported by recent phylogenetic analyses of chloroplast and mitochondrial genomes (Turmel et  
305 al. 2019). The computed ITS2 secondary structure of the new *Mantoniella* strains contained the

306 four helix domains found in many eukaryotic taxa (Supplementary Figure 4), in addition to Helix  
307 B9. The intramolecular folding pattern of the ITS2 transcript from *M. beaufortii* and  
308 *M. baffinensis* was very similar to the one from *M. squamata* and *M. antarctica* (Supplementary  
309 Figure 4). The universal hallmarks proposed by Mai and Coleman et al. (1997) and Schultz et al.  
310 (2005) were present in Helices II and III of the Mamiellaceae. These were the Y-Y (pyrimidine-  
311 pyrimidine) mismatch at conserved base pair 7 in Helix II (Figure 2) and YRRY (pyrimidine-  
312 purine-pyrimidine) motif at conserved positions 28–31 on the 5' side of Helix III  
313 (Supplementary Figure 5A). In all four strains, the Y-Y mismatch was represented by the pair U-  
314 U and the YRRY motif by the sequence UGGU.

315 The structural comparison at each base pair position within the ITS2 helices identified several  
316 compensatory base changes (CBCs) and single-side changes or hemi-CBCs (hCBCs), as well as  
317 conserved base pair positions among *Mantoniella* species (Supplementary Figure 4). Note that  
318 we only considered hCBCs at positions where the nucleotide bond was preserved. No CBCs  
319 were found between the three *M. beaufortii* strains consistent with their designation as a single  
320 species. However, three hCBCs were detected in Helix II at positions 15 and 17 (Figure 2) and  
321 Helix III at position 12 (Supplementary Figure 5A). Three CBCs were detected in Helices I  
322 (position 4), II (position 15) and IV (position 22) between *M. beaufortii* and *M. baffinensis*,  
323 supporting the separation of these strains into two distinct species (Figure 2 and Supplementary  
324 Figure 4). When possible, the evolutionary steps of the identified CBCs and hCBCs were  
325 mapped upon branches of the Mamiellaceae phylogenetic tree that was constructed based on the  
326 concatenated 18S/ITS2 (Figure 2 and Supplementary Figure 4) to distinguish synapomorphies  
327 from homoplasious changes (e.g. parallelisms and reversals). Few hypervariable positions  
328 showing several changes (CBCs and hCBCs) could not be unambiguously mapped upon the tree.



329

330 *Morphology and ultrastructure.* Under light microscopy, the cells of the new strains were green  
331 and round with one long and one short reduced flagellum ( $\sim 1 \mu\text{m}$ ), which were inserted almost  
332 perpendicularly to the cell (Figure 3). They swam with their flagella directed posteriorly, pushing  
333 the cell. Occasionally the cells ceased movement, pirouetted and took off again in a different  
334 direction (video links in the Materials and Methods). All strains possessed a stigma, visible in  
335 light microscopy as a red eyespot located opposite the flagella. Although there are no  
336 morphological characters that are unique to the mamiellophyceans and shared by all of its  
337 members, the new strains closely resembled *Mantoniella* and *Mamiella*, which are similarly  
338 small round bi-flagellated cells (see Supplementary Table 3 for morphological characters in  
339 described Mamiellophyceae). However, the flagella of *Mamiella* are of equal or near equal  
340 lengths (Moestrup 1984), so clearly the unequal flagella observed in our strains conform with  
341 described *Mantoniella* species, *M. squamata* and *M. antarctica* (Barlow and Cattolico 1980,  
342 Marchant et al. 1989). The new strains were thus morphologically indistinguishable by light  
343 microscopy from *Mantoniella* species, supporting their placement in the genus.

344 The new strains were in the size range (Table 2) reported for *M. squamata* (3–6.5  $\mu\text{m}$ ) and  
345 *M. antarctica* (2.8–5  $\mu\text{m}$ ) (Manton and Parke 1960, Marchant et al. 1989). Nonetheless,  
346 *M. beaufortii* strains were significantly smaller than *M. baffinensis* in cell diameter and average  
347 long flagellum length (Table 2) providing a means to distinguish the two new *Mantoniella*  
348 species from each other with light microscopy.

349 Transmission Electron Microscopy (TEM) of thin sections (Figure 4) and whole mounts  
350 (Figure 5) of the new strains provided details of their internal and external morphological  
351 features. The single chloroplast was cup-shaped with a pyrenoid surrounded by starch tubules

352 running through the pyrenoid. The stigma was composed of a single layer of oil droplets  
353 (approximately 0.1  $\mu\text{m}$  in diameter) (Figure 4A) and located at the periphery of the chloroplast  
354 facing the cell membrane, conforming to the description of the family Mamiellaceae (Marin and  
355 Melkonian 2010). Several large ejectosomes composed of fibrils were present at the cell  
356 periphery (Figure 4D and E). They are common in the Mamiellales (Moestrup 1984, Marchant et  
357 al. 1989) and are perhaps used to deter grazers.

358 One of the most salient features of the Mamiellophyceae is the presence of organic scales  
359 covering the cell, the most common of which comprise radiating and concentric ribs resembling  
360 spiderwebs that are present in the scale-bearing Mamiellales (*Bathycoccus*, *Mamiella* and  
361 *Mantoniella*), as well as *Dolichomastix* (Supplementary Table 3). We examined the whole  
362 mounts of the new *Mantoniella* species to establish the presence of scales and determine if they  
363 were morphologically distinguishable from related species, as *M. antarctica* (Marchant et al.  
364 1989) and *M. gilva* (Moestrup 1984) each have a unique type that differentiate them from other  
365 Mamiellales.

366 The flagella and cell bodies of the new strains were covered in imbricated spiderweb-like  
367 scales (Figure 5) measuring approximately 0.2  $\mu\text{m}$ . The scales were produced in the Golgi body  
368 (Figure 4B). The body scales were sub-quadrangular to oval whereas the flagellar scales were  
369 oval (Figure 5). Spiderweb scales had 6–8 major spokes radiating from the center with the  
370 number of spokes increasing towards the periphery and six or more concentric ribs dividing the  
371 scale into segments. In addition, there were some small scales (approximately 0.1  $\mu\text{m}$ ) on the cell  
372 body composed of four spokes (increasing to eight) and separated by four, more or less  
373 concentric, ribs (Figure 5D, G). The flagella were also covered by lateral hair scales, which were  
374 composed of two parallel rows of globular subunits. At the tip of the long flagellum there was a

375 tuft of three hair scales, for which the subunits were more closely packed together than the lateral  
376 hair scales (Figure 5). The hair scales of the new strains were identical to the "*Tetraselmis*-type"  
377 T-hairs previously described in *Mantoniella* and *Mamiella* (Marin and Melkonian 1994). This  
378 structure is otherwise only seen in *Dolichomastix lepidota* and differs from the smooth tubular T-  
379 hairs of *Dolichomastix tenuilepis* and *Crustomastix* (Marin and Melkonian 1994, Zingone et al.  
380 2002)(Supplementary Table 3).

381 Comparison of the spiderweb scales between *Mantoniella* species (Table 3) showed the new  
382 species differ significantly from *M. antarctica*, which possesses lace-like scales with six or seven  
383 radial ribs with very few concentric ribs (Marchant et al. 1989). Morphologically, the spiderweb  
384 scales of the new species most resembled *M. squamata*, which has large heptaradial flagellar  
385 scales, octaradial body scales and a few additional small tetraradial body scales (Marchant et al.  
386 1989). Indeed, the spiderweb scales of *M. baffinensis* (Figure 5) were structurally  
387 indistinguishable from *M. squamata*. In contrast, *M. beaufortii* shared the small tetraradial body  
388 scales but possessed hexaradial flagellar scales and heptaradial body scales, potentially allowing  
389 it to be differentiated from the other *Mantoniella* based on the number of radial spokes of the  
390 spiderweb scales.

391

392 *Pigment composition.* Pigment to chlorophyll *a* ratios of *M. beaufortii* RCC2288 were compared  
393 to a selection of other Chlorophyta species (Figure 6, Supplementary Table 4) from previous  
394 studies (Latasa et al. 2004, Lopes dos Santos et al. 2016), as pigments are useful phenotypic  
395 traits. Chlorophyll *a* and *b*, characteristics of Chlorophyta, were detected, as well as the basic set  
396 of carotenoids found in the prasinophytes: neoxanthin, violaxanthin, lutein, zeaxanthin,  
397 antheraxanthin and  $\beta$ -carotene. The additional presence of prasinoxanthin, micromonal and

398 uriolide placed RCC2288 in the PRASINO-3B group of prasinophyte green algae, *sensu* Jeffrey  
399 et al. (2011). This pigment-based grouping showed good agreement with the molecular  
400 phylogeny of Mamiellales, where the presence of prasinoxanthin, micromonal and the  
401 Unidentified M1 pigment are diagnostic of the order (Marin and Melkonian 2010). We did not  
402 detect Unidentified M1 in RCC2288, but as our analysis method differed from previous work  
403 (Latasa et al. 2004) and we relied on matching its chromatographic and spectral characteristics,  
404 its absence requires further confirmation. Notwithstanding, the pigment complement of  
405 RCC2288 was identical to other described Mamiellales (Figure 6, Supplementary Table 4),  
406 coherent with its classification within this order.

407 As noted by Latasa et al. (2004), Mamiellales pigment profiles are remarkably comparable  
408 (Figure 6), despite strains being cultured under very different conditions. Only a few carotenoids  
409 differed substantially (at least two fold) in relative abundance between *M. beaufortii* and the two  
410 other *M. squamata* strains analyzed: the concentration of neoxanthin, antheraxanthin and lutein  
411 were higher, whereas that of Mg-DVP and uriolide were relatively lower (Figure 6,  
412 Supplementary Table 4). Neoxanthin (associated with the light harvesting complex), as well as  
413 antheraxanthin and lutein (both involved in photoprotection), have previously been shown to  
414 increase significantly in *M. squamata* grown under continuous light compared to alternating  
415 light/dark cycles (Böhme et al. 2002). Therefore, the relatively high ratio of these carotenoids  
416 measured in *M. beaufortii* is consistent with growth under continuous light used with RCC2288.  
417 Uriolide and Mg-DVP have been observed to increase with light intensity in *M. squamata*  
418 (Böhme et al. 2002) and *Micromonas pusilla* (Laviale and Neveux 2011), respectively. Although  
419 more physiological data are required to interpret their relative decrease in RCC2288, these  
420 pigments are probably most responsive to light conditions (intensity and photoperiod).

421 Two unknown carotenoids were detected in RC2288, the first one having adsorption peaks at  
422 412, 436 and 464 nm, and the second one at 452 nm (Supplementary Table 5). These were  
423 relatively minor components comprising 2.7% and 1.5% of total carotenoids, respectively and  
424 may represent carotenoids unique to *M. beaufortii*.

425

426 *Environmental distribution.* In order to obtain information on the distribution of these two new  
427 species, we searched by BLAST both environmental GenBank sequences and published 18S V4  
428 and V9 metabarcode data sets (Supplementary Table 2). This allowed the retrieval of a few 18S  
429 rRNA sequences with higher than 98% similarity to the gene of RCC2288. Alignment of these  
430 sequences with other Mamiellophyceae sequences revealed diagnostic positions in both the V4  
431 and V9 hypervariable regions permitting *M. beaufortii* and *M. baffinensis* to be distinguished  
432 from other Mamiellophyceae, especially other *Mamiella* and *Mantoniella* species  
433 (Supplementary Figures 1 and 2). Signatures from the V4 region were clearer than from V9 due  
434 to the fact that for some of the strains, the sequences did not extend to the end of the V9 region  
435 (Supplementary Figure 2). In the V4 region, three signatures were observed, one common to  
436 both species (A in Supplementary Figure 1), while the other two (B and C in Supplementary  
437 Figure 1) differed between *M. beaufortii* and *baffinensis*.

438 No clone library or metabarcode sequences matched exactly *M. baffinensis*. In contrast, three  
439 environmental sequences (KT814860, FN690725, JF698785) from clone libraries had signatures  
440 similar to the *M. beaufortii* strains, two from Arctic Ocean water (Figure 7), including one  
441 obtained during the MALINA cruise, and one from ice originating from the Gulf of Finland. V4  
442 metabarcodes corresponding to *M. beaufortii* were found in the Ocean Sampling Day data set  
443 (Kopf et al. 2015) that includes more than 150 coastal samples at a single station off East

444 Greenland as well as in three metabarcoding studies in the Arctic Ocean, one in the Beaufort Sea  
445 performed during the MALINA cruise (Monier et al. 2015), one from Arctic sea ice (Stecher et  
446 al. 2016) where it was found at three stations and one from the White Sea (Belevich et al. 2017),  
447 also in the sea ice (Figure 7). No metabarcode corresponding to these two new species were  
448 found in waters from either the Southern Ocean or off Antarctica (Figure 7 and Supplementary  
449 Table 2). No metabarcodes from the V9 region corresponding to the two new species were found  
450 in the Tara Oceans data set that covered mostly temperate and subtropical oceanic regions (de  
451 Vargas et al. 2015). These data suggest that these species are restricted to polar Arctic regions  
452 (although we cannot exclude that they may be found in the future in the Antarctic which has  
453 been under-sampled until now) and are probably associated to sea ice although they can be  
454 present in the sea water, and that *M. beaufortii* is more wide spread than *M. baffinensis*.

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465

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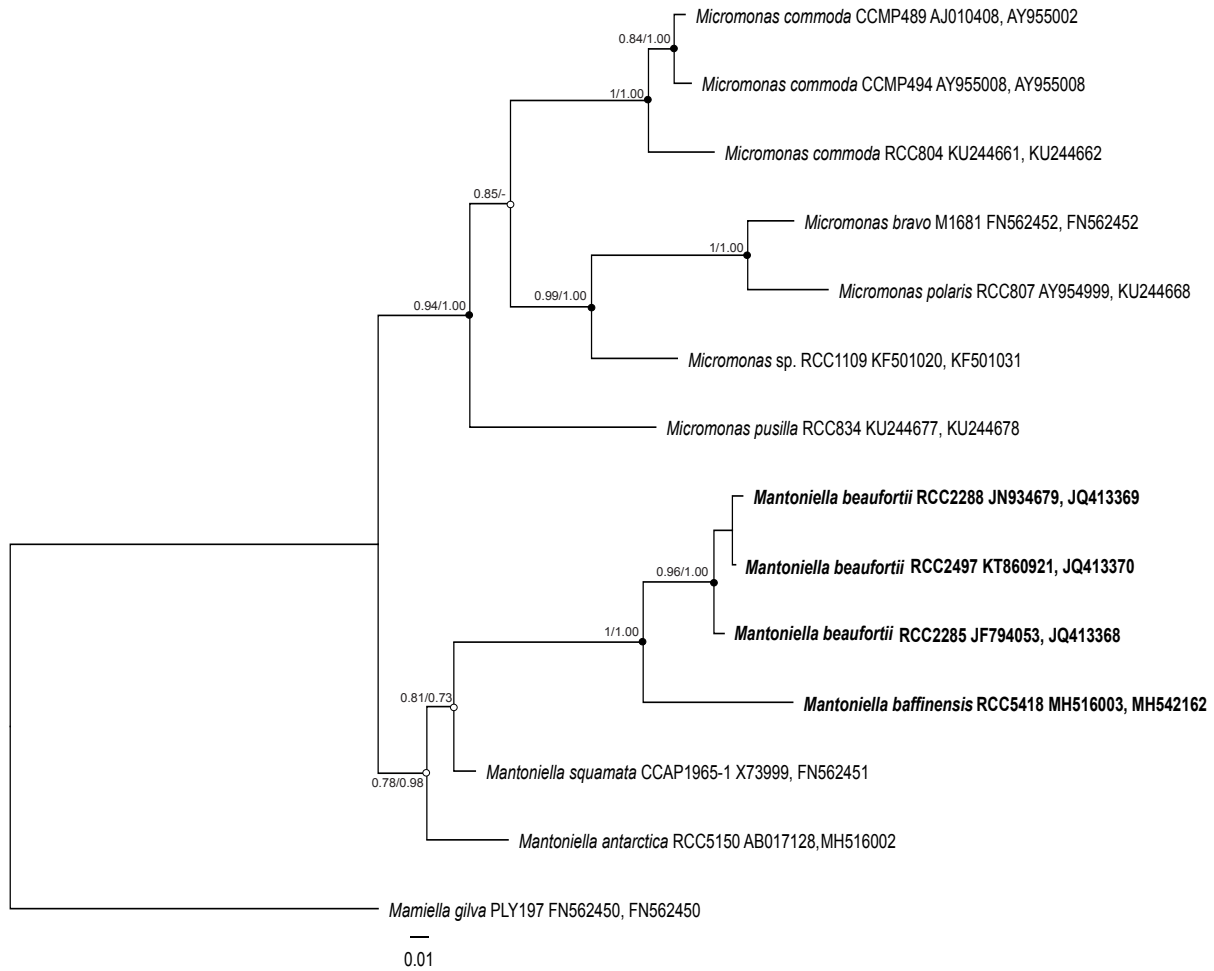
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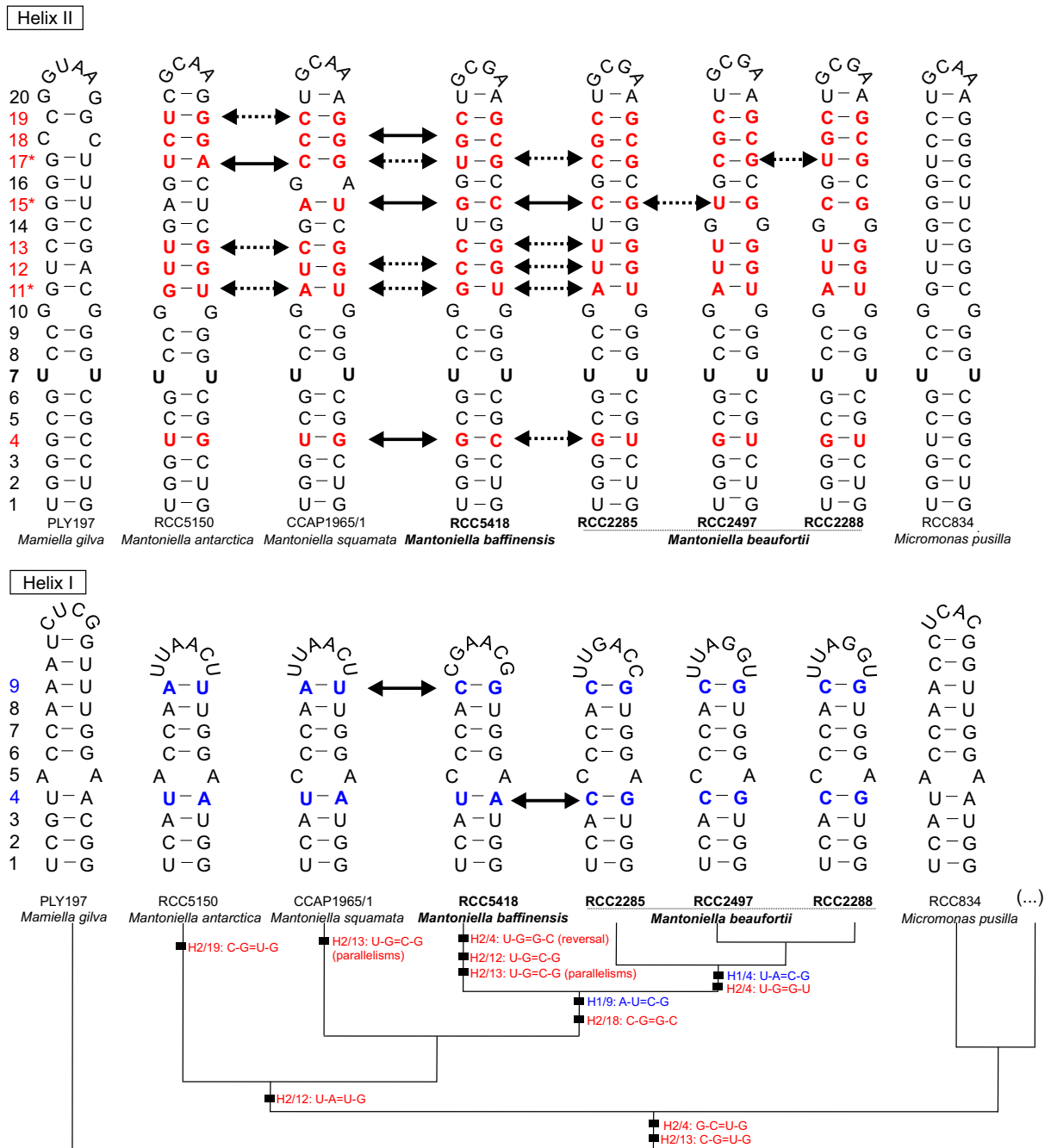
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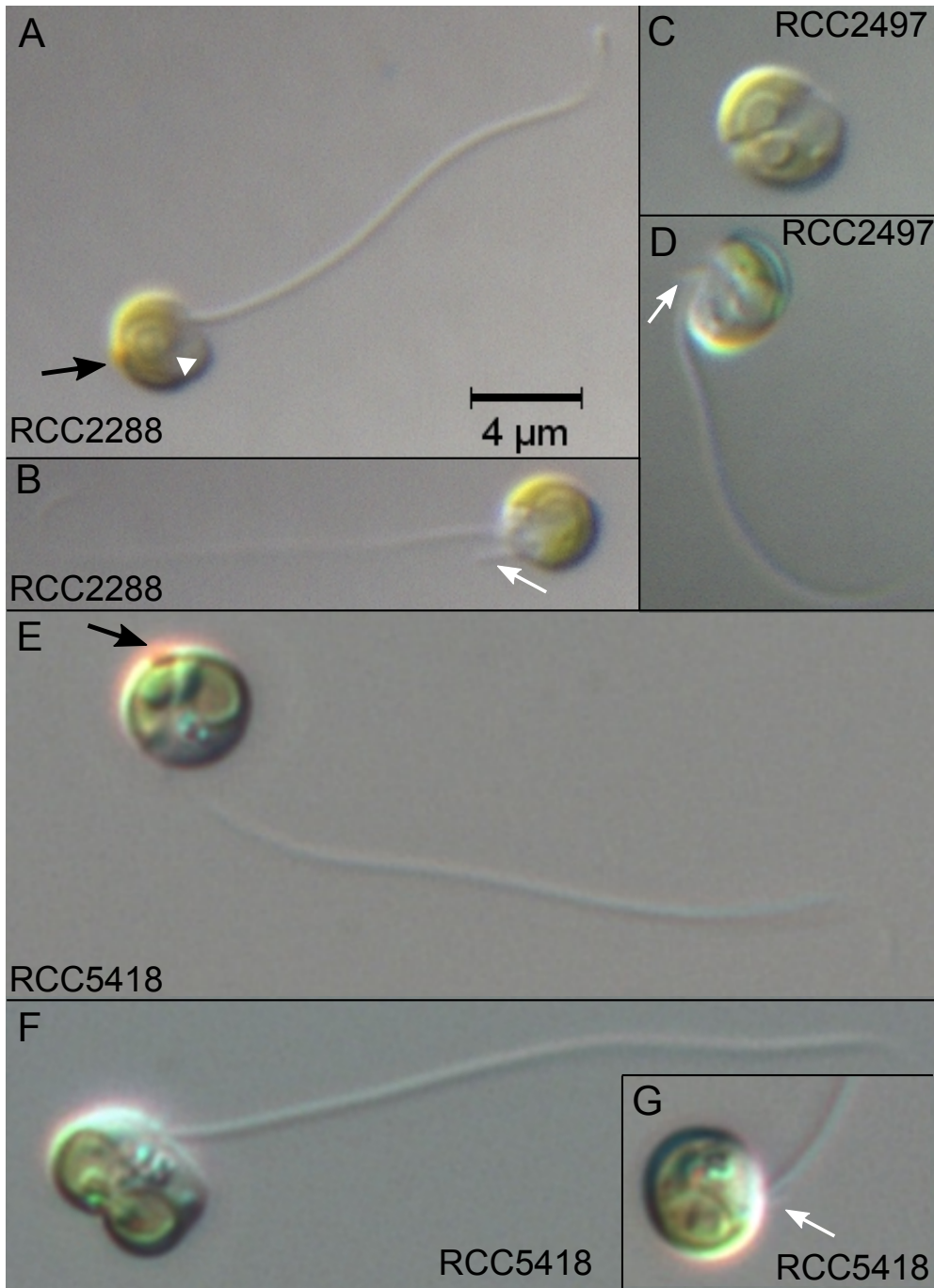
675 Figure 1. Maximum-likelihood tree inferred from concatenated 18S/ITS2 sequences of  
 676 Mamiellaceae. Solid dots correspond to nodes with significant support (> 0.8) for ML analysis  
 677 and Bayesian analysis (> 0.95). Empty dots correspond to nodes with non-significant support for  
 678 either ML or Bayesian analysis, or both.



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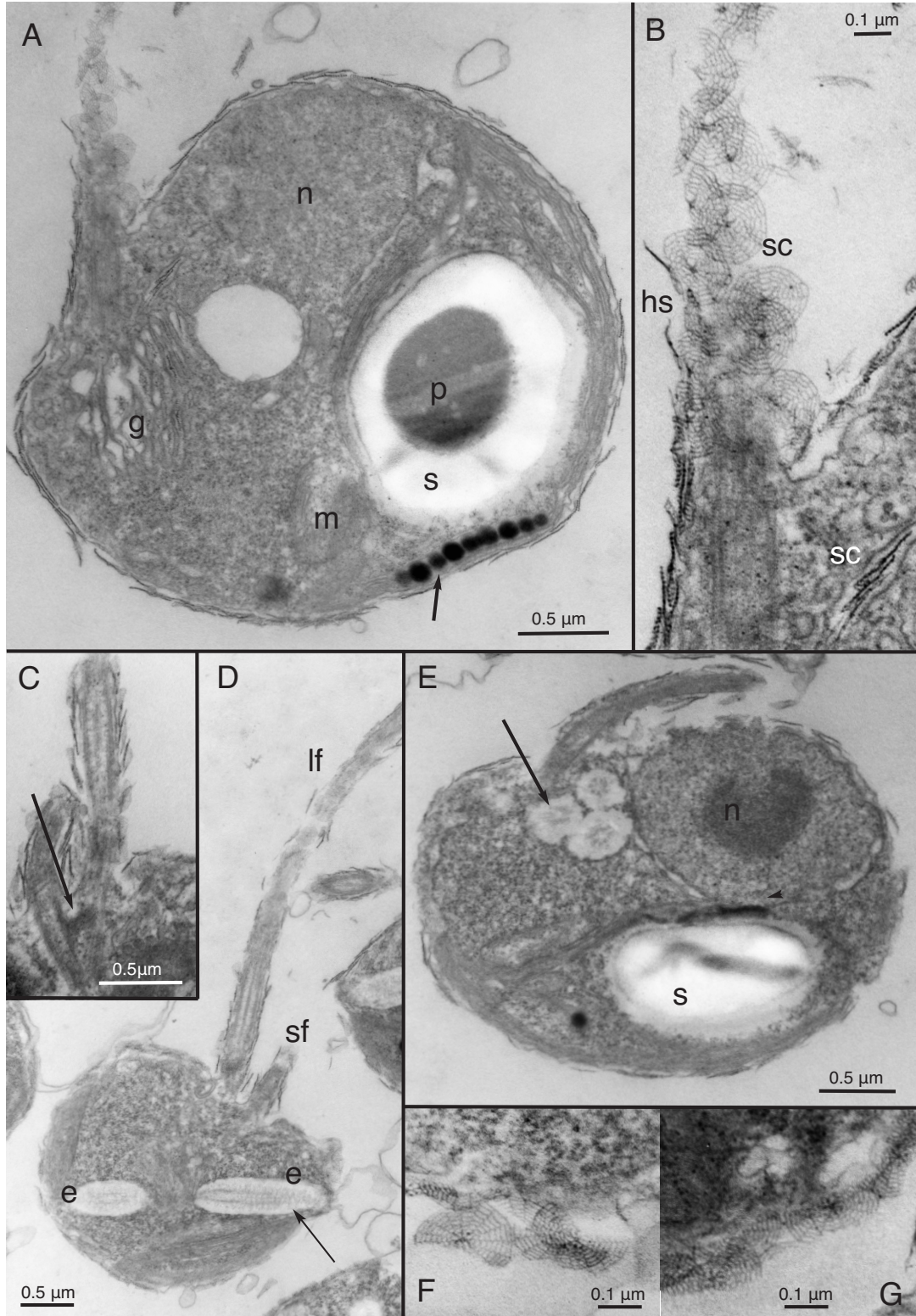
680 Figure 2. Molecular signatures of *Mantiella* species based on comparison of ITS2 secondary  
 681 structures within Mamiellaceae. Signatures in Helix I are shown in blue and Helix II in red. The  
 682 conserved base pairs among the different groups are numbered. Compensatory base changes  
 683 (CBCs) and hemi-CBCs (hCBCs) are highlighted by solid and dotted arrows respectively.

684 Hypervariable positions are marked by an asterisk (\*). Ellipsis (...) represent the other clades  
685 and species of *Micromonas*. The pyrimidine-pyrimidine (Y-Y) mismatch in Helix II is shown in  
686 bold black. Single nucleotide substitutions are shown by grey nucleotides. Identified  
687 homoplasious changes are shown as parallelisms and reversals.

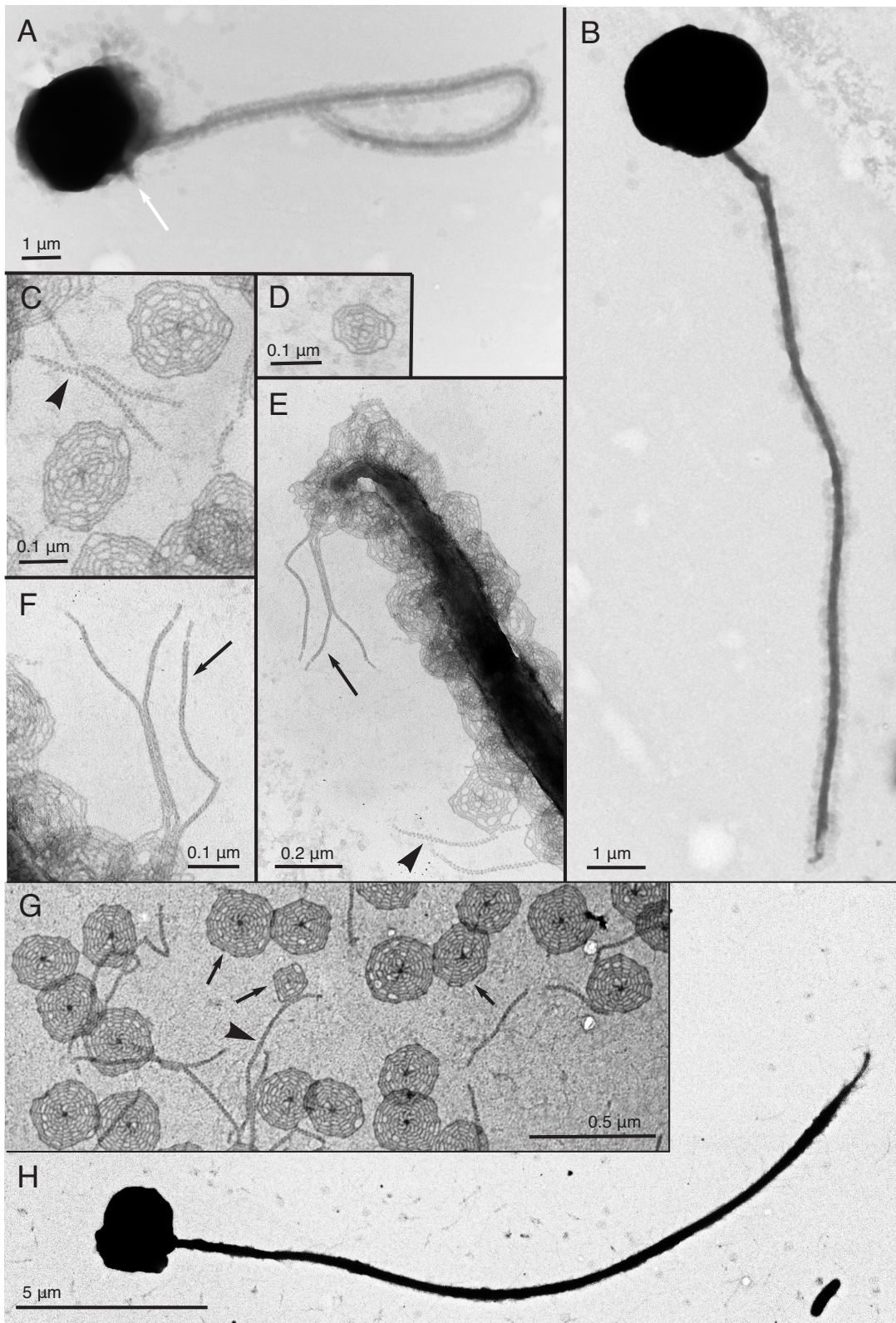


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689 Figure 3. Light microscopy images of the new *Mantoniella* strains. All strains have round cell  
690 morphology, visible red stigma (black arrow), a long and short flagellum (white arrow) and one  
691 chloroplast with a pyrenoid (white arrowhead). Scale bar is 4  $\mu\text{m}$  for all images. (A–B)  
692 *M. beaufortii* RCC2288. (C–D) *M. beaufortii* RCC2497 during cell division and single cell  
693 showing long and short flagellum. (E–G) *M. baffinensis* RCC5418 single cell (E), during cell  
694 division (F) and cell showing the short flagellum (G inset).

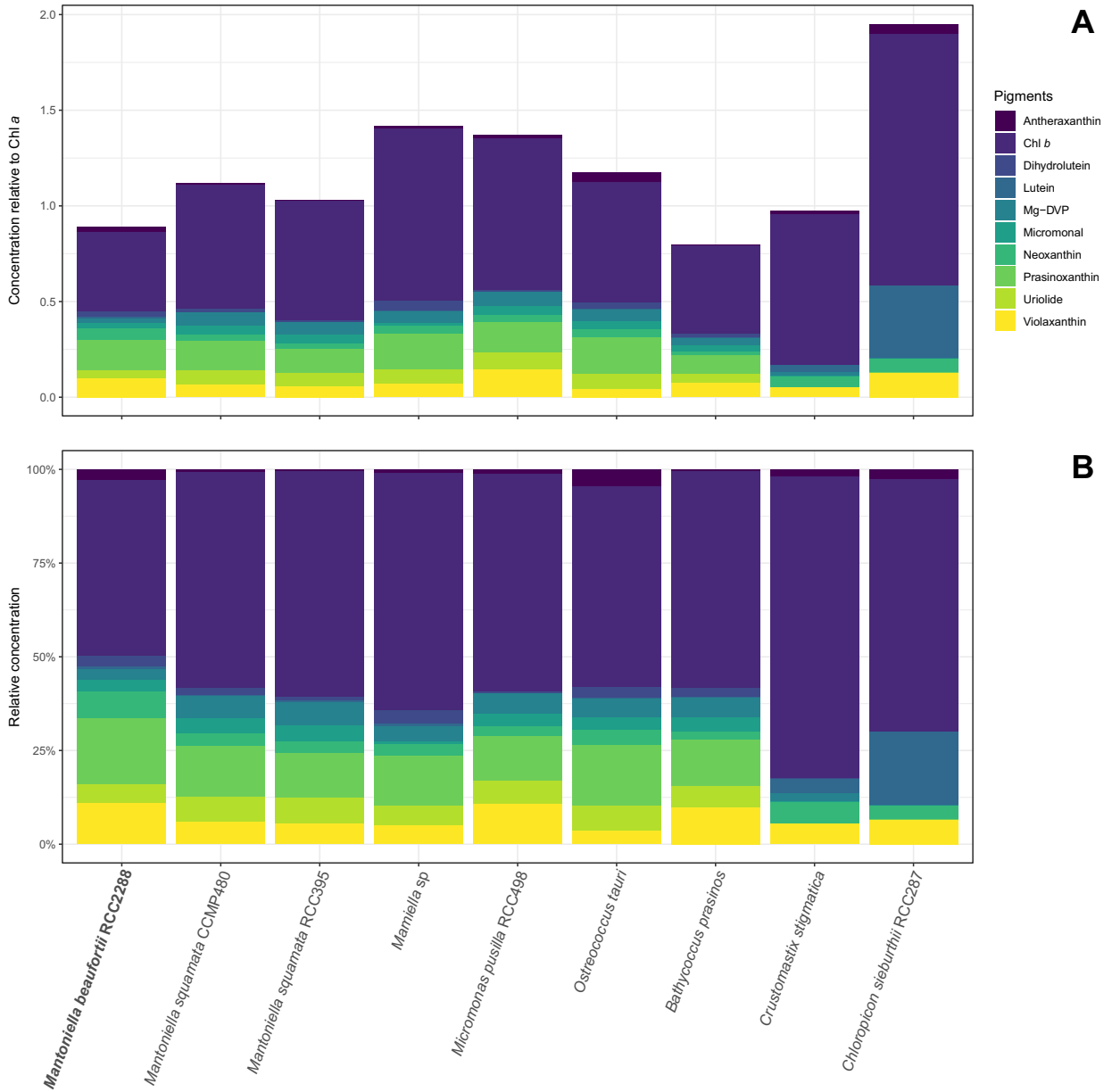


696 Figure 4. TEM thin sections of *M. beaufortii* RCC2288. (A) Internal cell structure showing  
697 organelles and stigma (black arrow). (B) Detail of the hair and spiderweb scales covering the  
698 long flagellum. Scales produced in the Golgi body. (C) Detail of the flagellar base (black arrow).  
699 (D) Cell with long and short flagella and longitudinal section of the ejectosomes (black arrow).  
700 (E) Cross section of ejectosomes (black arrow). (F) and (G) body scales made up of radiating  
701 and concentric ribs. Abbreviations: e=ejectosome, g=Golgi, s=starch granule, m=mitochondrion,  
702 n=nucleus, p=pyrenoid, hs=hair scale, sc=scale, lf=long flagellum and sf=short flagellum.



704 Figure 5. Transmission electron micrographs of whole-mounts of the new *Mantoniella* strains.  
705 (A–E) *M. beaufortii*. (A) Whole cells of strain RCC2288, indicating the short flagellum (white  
706 arrow), and (B) RCC2497. (C) Detached flagellar spiderweb-like scales and hair scales (black  
707 arrowhead). (D) Detail of small tetradial body scale. (E) Imbricated scales and hair scales  
708 covering the long flagellum. A tuft of three hair scales on the tip of the long flagellum (black  
709 arrow) (F) Detail of the tuft of hair scales (black arrow). (G–H) *M. baffinensis* RCC5418. (G)  
710 Small and large body scales (black arrows) and flagellar hair scales (black arrowhead) and (H)  
711 whole cell.





712

713 Figure 6. Pigment to chlorophyll *a* ratios in *M. beaufortii* RCC2288 (this study) compared to

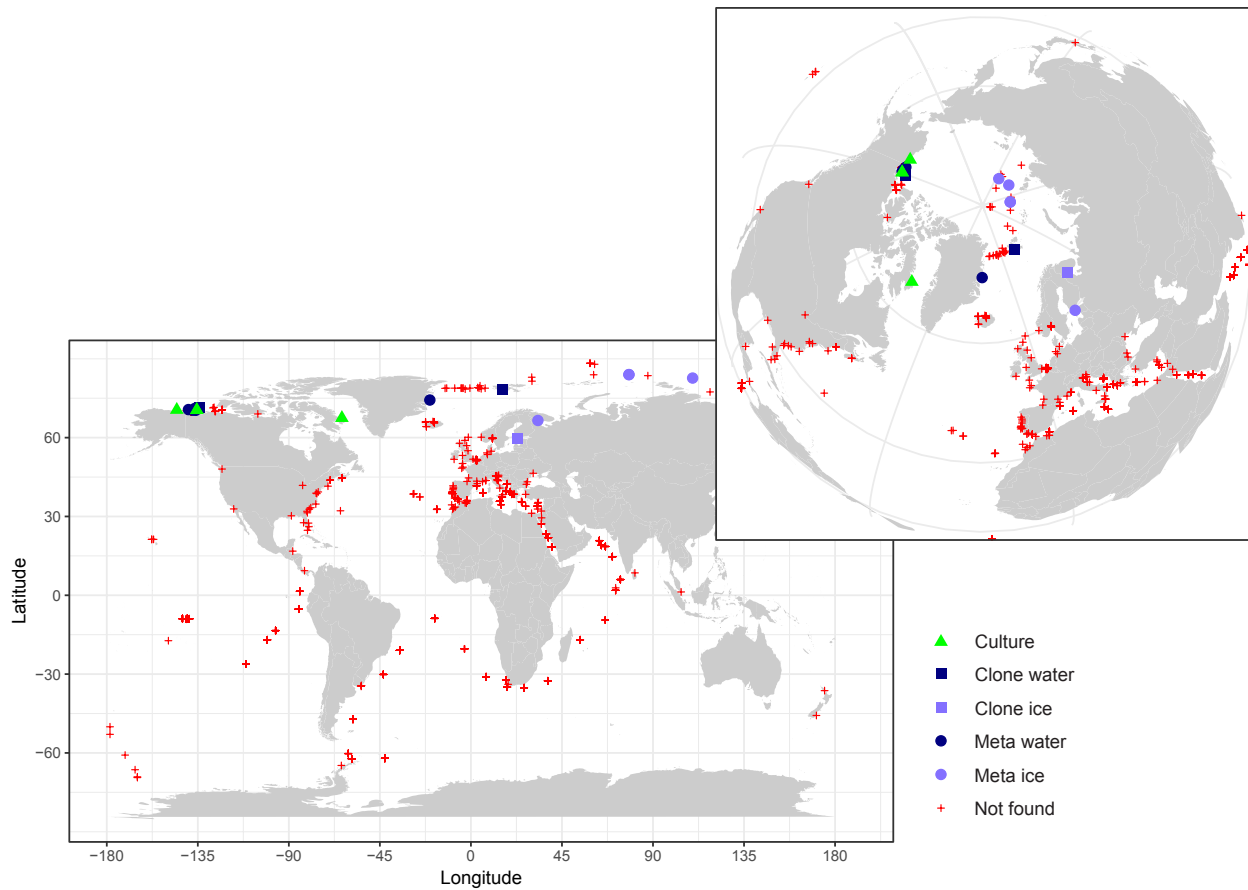
714 other Mamiellophyceae species (data from Latasa et al. 2004). (A) Cumulative pigment to

715 Chlorophyll *a* ratio of Chlorophyll *b* and abundant carotenoids (excluding  $\alpha$ - and  $\beta$ -carotene).

716 (B) As for A, but showing relative abundances. Mg-DVP: Mg-24-divinyl pheoporphyrin *a*5

717 monomethyl ester.

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719

720 Figure 7. Map of the distribution of *M. beaufortii* in environmental sequence datasets  
 721 highlighting its prevalence in Arctic samples (inset). The isolation sites of *M. beaufortii* cultures,  
 722 presence of its 18S rRNA gene sequence in clone libraries (Clone water, Clone ice) and  
 723 metabarcodes from seawater and ice samples (Meta water, Meta ice) and absence in  
 724 metabarcodes (Not found) are plotted. For *M. baffinensis*, only its isolation site is indicated in  
 725 Baffin Bay since no similar environmental sequence was found in the datasets analyzed.  
 726 Metabarcoding datasets include Ocean Sampling Day, Tara Oceans and polar projects. See  
 727 Supplementary Table 2 for a full description of the metabarcoding datasets screened.

728

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730

731 *Tables*

732 Table 1. Strains used in this study. RCC: Roscoff Culture Collection ([www.roscoff-culture-](http://www.roscoff-culture-collection.org)  
 733 [collection.org](http://www.roscoff-culture-collection.org)). 18S rRNA and ITS show Genbank accession numbers. Strains in bold used to  
 734 describe the new species.

Strain	Strain Name	Oceanic Region	Latitude	Longitude	Depth of Isolation (m)	18S rRNA	ITS	Remark
RCC2285	MALINA E43.N1	Beaufort Sea	70° 34' N	145° 24' W	0	JF794 053	JQ413368	strain lost
RCC2288	MALINA E47.P2	Beaufort Sea	70° 30' N	135° 30' W	0	JN934 679	JQ413369	
RCC2497	MALINA E47.P1	Beaufort Sea	70° 30' N	135° 30' W	0	KT860 921	JQ413370	
RCC5418	GE_IP_IC_DIL_490	Baffin Bay	67° 28' N	63° 46' W	surface ice	MH51 6003	MH542162	

735

736 Table 2. Cell diameter and long flagellum lengths measured for *M. beaufortii* (RCC2288 and  
 737 RCC2497) and *M. baffinensis* (RCC5418). n = number of cells measured and SD = standard  
 738 deviation.

Strain	min	max	mean	median	stdev	n
<b>Cell diameter (µm)</b>						
RCC2288	2.89	4.98	3.77	3.70	0.41	60
RCC2497	3.15	4.74	3.87	3.77	0.39	39
RCC5418	3.54	5.69	4.66	4.66	0.51	69
<b>Long flagellum length (µm)</b>						
RCC2288	12.93	21.47	16.27	15.99	2.63	11
RCC2497	11.91	21.25	16.31	17.07	2.71	12
RCC5418	11.27	32.59	21.78	21.29	5.14	25

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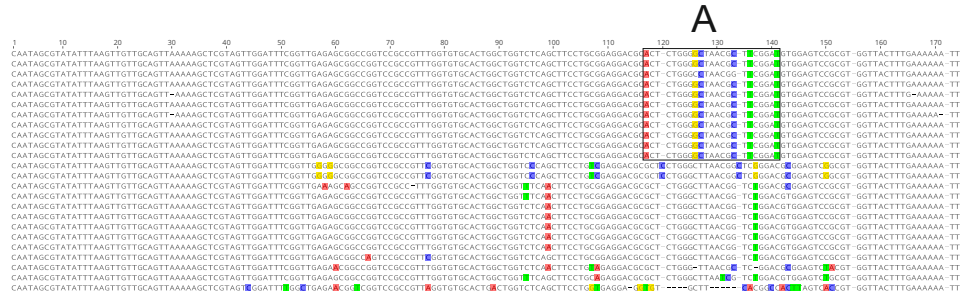
740 Table 3. Comparison of *Mantoniella* spp. scale types.

Species	Flagellar scales	Body scales
<i>Mantoniella squamata</i>	spiderweb-like heptaradial	spiderweb-like large octoaradial and small rare tetradial
<i>Mantoniella antarctica</i>	lace-like heptaradial	lace-like hexaradial and smaller heptaradial
<i>Mantoniella beaufortii</i>	spiderweb-like hexaradial	spiderweb-like large heptaradial and small rare tetradial
<i>Mantoniella baffinensis</i>	spiderweb-like heptaradial	spiderweb-like large octoaradial and small rare tetradial

741

742 *Supplementary Figures*

**Mantiella beaufortii** RCC2288 - JN934679  
**Mantiella beaufortii** RCC2497 - K1860921  
**Mantiella beaufortii** RCC2285 - JF794053  
**Baltic Sea** - clone 4-E5 - FN690725  
**Isfjorden, West Spitsbergen** - clone 34c 7185 - K7814860  
**Beaufort Sea** clone MALINA\_S1320\_3m\_Nano\_E5069\_D8 - JF698785  
**MALINA\_Monier\_2014** otu414  
**Arctic Ocean Stecher 2016** gnl[SRA]ERR660653.5901.3  
**Land-Fast ice of the White Sea - OTU\_1\_14\_10 - MF589928**  
**OSD LGC - V4** otu4099  
**Mantiella baffinensis** RCC5418 - MH516003  
**Mamiella glava strain PLY 197** - FN562450  
**Mamiella sp. - AB017129**  
**Mantiella antbeaufortii** - AB017128  
**Mantiella squamata isolate K-0284** - KU600447  
**Beaufort Sea** clone CFL1460B03 - HM561186  
**Wolf 2014 - South Pacific Ross Sea** gnlSR815917.205358.2  
**Mantiella squamata - X73999**  
**Land-Fast ice of the White Sea - OTU\_1\_14\_28 - MF589927**  
**Mantiella sp. strain MNURJ08** - K781060  
**Taiwan - coastal** clone 08803P04 - KU743480  
**Micromonas pusilla** CCMF2099 - DQ025753  
**Micromonas pusilla** RCC692 - K7860893  
**Ostreococcus tauri** genome 18S rRNA



**Mantiella beaufortii** RCC2288 - JN934679  
**Mantiella beaufortii** RCC2497 - K1860921  
**Mantiella beaufortii** RCC2285 - JF794053  
**Baltic Sea** - clone 4-E5 - FN690725  
**Isfjorden, West Spitsbergen** - clone 34c 7185 - K7814860  
**Beaufort Sea** clone MALINA\_S1320\_3m\_Nano\_E5069\_D8 - JF698785  
**MALINA\_Monier\_2014** otu414  
**Arctic Ocean Stecher 2016** gnl[SRA]ERR660653.5901.3  
**Land-Fast ice of the White Sea - OTU\_1\_14\_10 - MF589928**  
**OSD LGC - V4** otu4099  
**Mantiella baffinensis** RCC5418 - MH516003  
**Mamiella glava strain PLY 197** - FN562450  
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**Mantiella squamata - X73999**  
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**Micromonas pusilla** CCMF2099 - DQ025753  
**Micromonas pusilla** RCC692 - K7860893  
**Ostreococcus tauri** genome 18S rRNA



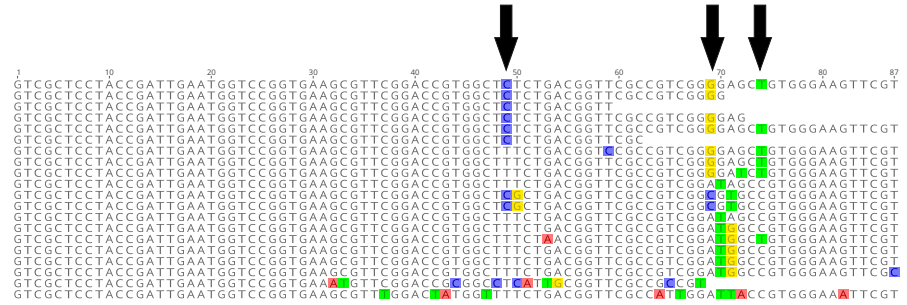
743  
 744 **Supplementary Figure 1. Alignment of the 18S rRNA gene V4 hypervariable region from**  
 745 ***M. beaufortii* and *M. baffinensis* strains (Red and Orange, respectively), environmental clones**  
 746 **(Blue) and metabarcodes (Green) with a selection of sequences from closely related**  
 747 **Mamiellophyceae. Sequence signatures diagnostic of the two new species are indicated by boxes.**  
 748 **The A region is specific of both species while the B and C regions differ between the two**  
 749 **species.**

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**Mantoniella beaufortii** RCC2497 - KT860921  
**Mantoniella beaufortii** RCC2288 - Jn934679  
**Mantoniella beaufortii** RCC2285 - Jf794053  
**Mantoniella baffinensis** RCC3418 - Mh516003  
**Baltic Sea - clone 4-E5 - FN690725**  
**Beaufort Sea - clone MALINA S1320 3m - Nano E5069 D8 - JF698785**  
North Pole - clone North\_Pole\_S1120\_29 - HQ438123  
Baltic Sea sea ice - clone S-D5 - FN690723  
Baltic Sea sea ice - clone 4-D6 - FN690722  
V9 Tara\_866d68b573bb1042852502405760a7d  
Mamiella giva strain PLY 197 - FN562450  
Mamiella sp. from Shizugawa, Miyagi, Japan - AB017129  
V9 Amaral Antarctic gni | SRA | SR0119554.11544.2  
V9 Framaren 2009 Stoeck gni | SRA | SR027232.6090.2  
Mantoniella antarctica from Lutzow Holmbukta, Antarctica - AB017128  
Mantoniella squamata isolate K-0284 - KU600447  
Mantoniella squamata strain CCAP 1965/1 - X79999  
Taiwan coastal - clone 08B03P04 - KU743480  
Micromonas pusilla RCC692 - KT860893  
Ostreococcus tauri genome



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755 Supplementary Figure 2. Alignment of the 18S rRNA gene V9 hypervariable region from

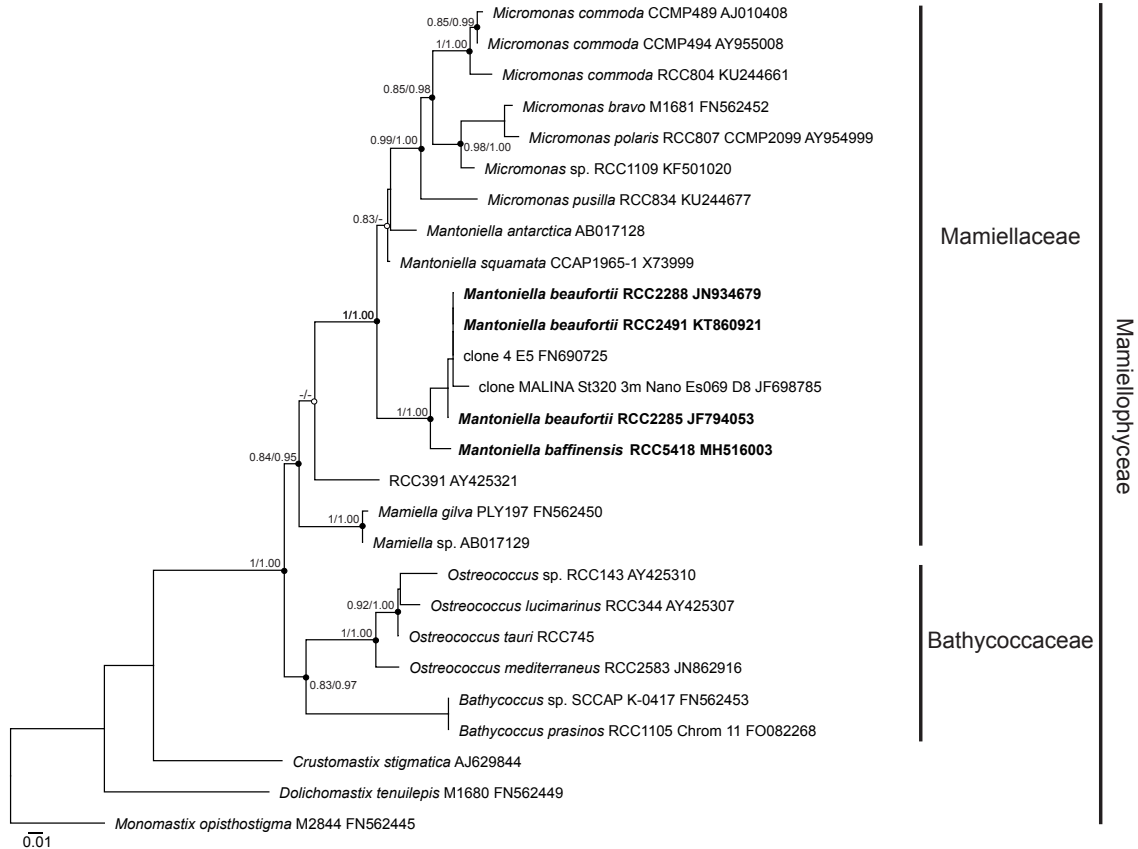
756 *M. beaufortii* and *M. baffinensis* strains (Red and Orange, respectively) and environmental

757 clones (blue) with a selection of closely related Mamiellophyceae sequences. Sequence

758 signatures diagnostic of *M. beaufortii* and *M. baffinensis* are indicated by arrows.

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762 Supplementary Figure 3. Maximum-likelihood phylogenetic tree inferred from nuclear 18S

763 rRNA sequences of Mamiellophyceae. *Monomastix opisthostigma* was used as an outgroup.

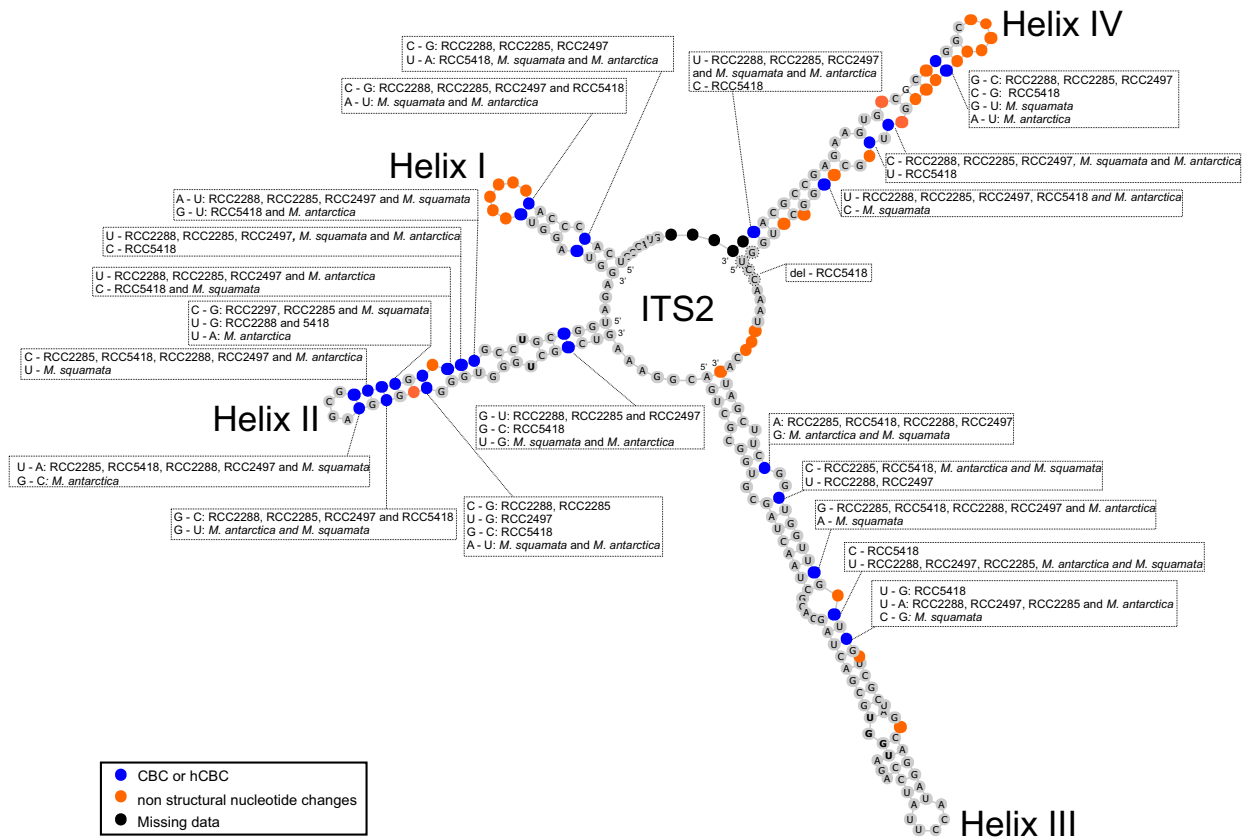
764 Solid dots correspond to nodes with significant support (> 0.8) for ML analysis and Bayesian

765 analysis (>0.95). Empty dots correspond to nodes with non-significant support for either ML or

766 Bayesian analysis, or both. GenBank accessions of the 18S rRNA sequences shown after the

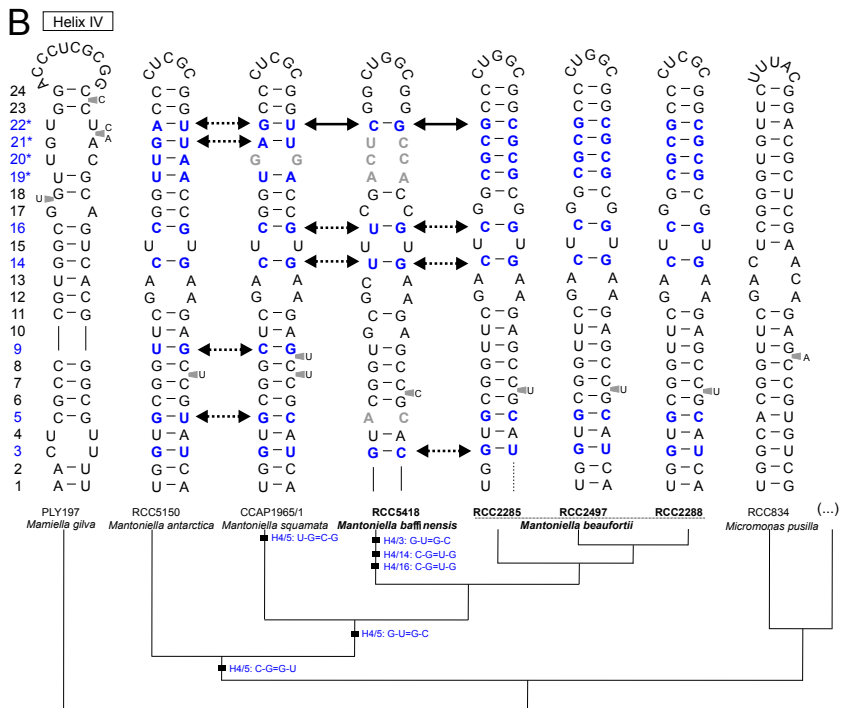
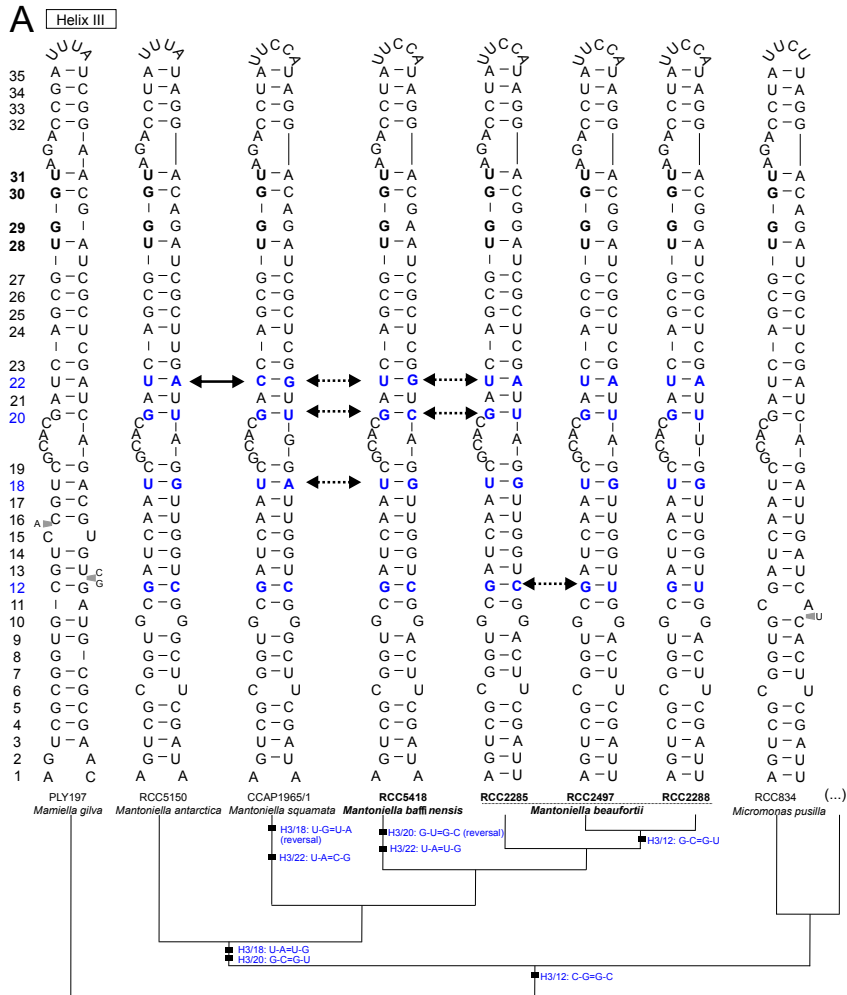
767 species name.

768



769

770 Supplementary Figure 4. Intramolecular folding pattern of the ITS2 molecule of *Mantoniella*  
 771 (RCC2288, RCC2285, RCC2497 and RCC5418). The four major helices are labeled as Helix I –  
 772 Helix IV. Blue dots represent either CBCs or hCBCs. Non-CBCs (N – N ↔ N × N) are  
 773 represented in orange.





775 Supplementary Figure 5. Molecular signatures of *Mantoniella* species revealed by comparison of  
776 ITS2 secondary structures within Mamiellaceae. Signatures in Helix III are shown in (A) and  
777 Helix IV in (B). The conserved base pairs among the different groups are numbered. CBCs and  
778 hCBCs are highlighted by solid and dotted arrows, respectively. Hypervariable positions are  
779 marked by an asterisk (\*). Ellipsis (...) represent the other clades and species of *Micromonas*.  
780 The YRRY (pyrimidine-purine-pyrimidine) motif on the 5' side arm of Helix III is shown in  
781 bold black. Single nucleotide substitutions are shown by grey nucleotides. Identified  
782 homoplasious changes are shown as parallelisms and reversals.  
783  
784 *Supplementary Tables*

785

786 Supplementary Table 1. Primers and PCR conditions used in this study. Abbreviations: fwd - forward, rev. - reverse, temp -  
 787 temperature.

Gene	Primer fwd	Sequence 5'-3'	Primer rev.	Sequence 5'-3'	Reference	cycles	Initial time	Denaturation temp	Denaturation time	temp	Annealing time	Annealing temp	Extension time	Extension temp	Elongation time	Elongation temp
18S rRNA	Euk63F	ACGCTTGT CTCAAAGA TTA	Euk1818R	ACGGAAA CCTTGTTA CGA	Lepère et al. 2011	35	5 min	95°C	30 sec	95°C	30 sec	55°C	1 min 30 sec	72°C	10 min	72°C
ITS1-5.8S-ITS2	329f	GTGAACCT GCRGAAGG ATCA	D1R-R	TATGCTTA AATTCAGC GGGT	Balzano et al. 2017	35	5 min	95°C	1 min	95°C	45 sec	55°C	1 min 15 sec	72°C	7 min	72°C

788 Supplementary Table 2. Metabarcoding datasets of the 18S rRNA gene analyzed in this study for  
 789 the presence of *M. beaufortii* and *M. baffinensis* signatures.

Data set	18S region	Region	Samples	Bioproject or link	Sequencer	Clustering	Reference
Tara Oceans	V9	Oceanic	334	<a href="http://doi.org/10.1594/PANGAEA.843022">http://doi.org/10.1594/PANGAEA.843022</a>	Illumina	Swarm	de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahe, F., Logares, R., et al. (2015). Eukaryotic plankton diversity in the sunlit ocean. <i>Science</i> 348, 1261605. doi:10.1126/science.1261605.
OSD - LGC - 2014	V4	Coastal	157	PRJEB8682	Illumina	0.97	Kopf, A., Bick, M., Kottmann, R., Schnetzer, J., Kostadinov, I., Lehmann, K., Fernandez-Guerra, A. et al. 2015. The ocean sampling day consortium. <i>Gigascience</i> . 4:27.
MALINA - Monier - 2014	V4	Arctic Ocean	24	PRJNA202104	454	0.98	Monier, A., Terrado, R., Thaler, M., Comeau, A., Medrinal, E. & Lovejoy, C. 2013. Upper Arctic Ocean water masses harbor distinct communities of heterotrophic flagellates. <i>Biogeosciences</i> . 10:4273–86. Monier, A., Comte, J., Babin, M., Forest, A., Matsuzaka, A. & Lovejoy, C. 2014. Oceanographic structure drives the assembly processes of microbial eukaryotic communities. <i>ISME J.</i> 9:990–1002.
ACME - Comeau - 2011	V4	Arctic Ocean	11	SRA029114	454	0.98	Comeau, A. M., Li, W. K. W., Tremblay, J.-É., Carmack, E. C. & Lovejoy, C. Arctic Ocean microbial community structure before and after the 2007 record sea ice minimum. <i>PLoS One</i> 6, e27492 (2011)
Nansen Basin - Metfies - 2016	V4	Arctic Ocean	17	PRJEB11449	454	0.97	Metfies, K., von Appen, W.-J., Kiliyas, E., Nicolaus, A. & Nöthig, E.-M. Biogeography and Photosynthetic Biomass of Arctic Marine Pico-Eukaryotes during Summer of the Record Sea Ice Minimum 2012. <i>PLoS One</i> 11, 20 pp. (2016)
Southern Ocean - Wolf - 2014	V4	Southern Ocean	6	PRJNA176875	454	0.97	Wolf, C., Frickenhaus, S., Kiliyas, E. S., Peeken, I. & Metfies, K. Protist community composition in the Pacific sector of the Southern Ocean during austral summer 2010. <i>Polar Biol.</i> 37, 375–389 (2014)
Fildes Bay - Luo - 2016	V4	Southern Ocean	10	PRJNA254097	Illumina	0.97	Luo, W. et al. Molecular diversity of microbial eukaryotes in sea water from Fildes Peninsula, King George Island, Antarctica. <i>Polar Biol.</i> (2015). doi:10.1007/s00300-015-1815-8
Fram Strait - Kiliyas - 2013	V4	Arctic Ocean	5		454	0.97	Kiliyas, E., Wolf, C., Nöthig, E.-M., Peeken, I. & Metfies, K. Protist distribution in the Western Fram Strait in summer 2010 based on 454-pyrosequencing of 18S rDNA. <i>J. Phycol.</i> 49, 996–1010 (2013).

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802 Supplementary Table 3. Morphological characters in Mamiellophyceae species.

Species	Flagella	Spider web-like scales	Circular patterned scales	T-hair scales: tubular shaft	T-hair scales: globular subunits
<i>Bathycoccus prasinus</i>	no	+	-	-	-
<i>Ostreococcus tauri</i>	no	-	-	-	-
<i>Ostreococcus mediterraneus</i>	no	-	-	-	-
<i>Mamiella gilva</i>	2 long	+	-	-	+
<i>Mantoniella squamata</i>	1 long, 1 short	+	-	-	+
<i>Mantoniella antarctica</i>	1 long, 1 short	+	-	-	+
<i>Mantoniella beaufortii</i>	1 long, 1 short	+	-	-	+
<i>Mantoniella baffinensis</i>	1 long, 1 short	+	-	-	+
<i>Micromonas pusilla</i>	1 long, 2 extending microtubules	-	-	-	-
<i>Micromonas commoda</i>	1 long, 2 extending microtubules	-	-	-	-
<i>Micromonas polaris</i>	1 long, 2 extending microtubules	-	-	-	-
<i>Micromonas bravo</i>	1 long, 2 extending microtubules	-	-	-	-
<i>Crustomastix didyma</i>	2 long	-	-	+	-
<i>Crustomastix stigmatica</i>	2 long	-	-	+	-
<i>Dolichomastix eurylepidea</i>	2 long	+	-	?	?
<i>Dolichomastix lepidota</i>	2 long	-	-	-	+
<i>Dolichomastix nummulifera</i>	2 long	-	+	?	?
<i>Dolichomastix tenuilepsis</i>	2 long	+	+	+	-

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811 Supplementary Table 4. Pigment composition of *M. beaufortii* (RCC2288) compared to a  
 812 selection of green algae. Values are shown as a ratio of pigment to Chl *a* concentration and  
 813 percent contribution to total carotenoids (in italics). See Supplementary Table 5 for the full  
 814 names of the pigments.

Species	Order	Phyt n a	Chl d a	Chl b	Mg- DVP	%	Uri	%	Neo	%	Pra	%	Vio	%	Mnna l	%	Ant	%	Lut	%	Dihy	%	<i>α</i> -car + <i>β</i> -car	%
<b><i>M. beaufortii</i></b> RCC2288	Mamiellales	0.006	0.012	0.416	0.024	3.6	0.045	6.6	0.063	9.4	0.156	23.2	0.099	14.6	0.028	4.2	0.025	3.7	0.006	0.9	0.027	4.0	0.167	24.8
<i>M. squamata</i> CCMP480*	Mamiellales	ND	ND	0.644	0.068	10.1	0.075	11.1	0.04	5.5	0.15	22.2	0.068	10.1	0.045	6.7	0.006	0.9	0.003	0.4	0.021	3.1	0.121	17.9
<i>M. squamata</i> RCC395*	Mamiellales	ND	ND	0.62	0.065	12.2	0.073	13.7	0.03	5.8	0.123	23.1	0.056	10.5	0.043	8.1	0.003	0.6	0.004	0.8	0.009	1.7	0.062	11.7
<i>O. tauri</i> RCC116*	Mamiellales	ND	ND	0.633	0.061	8.6	0.079	11.1	0.05	6.3	0.191	26.9	0.044	6.2	0.039	5.5	0.051	7.2	0.003	0.4	0.032	4.5	0.098	13.8
<i>B. prasinos</i> RCC113*	Mamiellales	ND	ND	0.462	0.041	9.7	0.047	11.1	0.02	4.3	0.097	22.9	0.078	18.4	0.03	7.1	0.002	0.5	0.003	0.7	0.018	4.3	0.029	6.9
<i>M. pusilla</i> CCMP490*	Mamiellales	ND	ND	0.768	0.072	9.7	0.096	13.0	0.05	6.1	0.191	25.8	0.103	13.9	0.053	7.2	0.019	2.6	0.006	0.8	0.01	1.3	0.058	7.8
<i>Mamiella</i> sp. RCC391*	Mamiellales	ND	ND	0.896	0.060	8.7	0.072	10.5	0.04	6.3	0.189	27.6	0.074	10.8	0.01	1.5	0.011	1.6	0.008	1.2	0.052	7.6	0.082	12.0
<i>C. stigmatica</i> *	Dolichomastigales	ND	ND	0.786	0.023	3.5	0	0	0.06	8.4	0	0	0.055	8.3	0	0	0.017	2.6	0.038	5.7	0	0	0.305	45.9
<i>C. sieburthii</i> RCC287†	Chloropicales	ND	0.106	0.986	0	0	0	0	0.17	14.8	0	0	0.572	50.8	0	0	0.024	2.1	0.363	32.2	0	0	-	-
<i>P. parkeae</i> CCMP724*	Pyramimonadales	ND	ND	0.677	0.001	0.4	0	0	0	0	0	0	0.077	27.7	0	0	0.002	0.7	0.07	25.2	0	0	0.088	31.7
<i>N. pyriformis</i> CCMP717*	Nephrodelmidae	ND	ND	0.698	0.015	3.5	0	0	0.02	5.0	0	0	0.077	18.2	0	0	0.016	3.8	0.022	5.2	0	0	0.065	15.3
<i>Tetraselmi</i> sp. RCC234*	Chlorodendrales	ND	ND	0.812	0.022	4.6	0	0	0.03	6.8	0	0	0.057	11.8	0	0	0.006	1.2	0.096	19.9	0	0	0.082	17.0
<i>P. marina</i> *	Pseudoscourfieldiales	ND	ND	0.871	0.071	13.3	0	0	0.06	10.7	0.287	53.8	0.036	6.8	0	0	0	0	0.003	0.6	0	0	0.033	6.2
<i>P. capsulatus</i> CCMP1192*	Prasinococcales	ND	ND	0.62	0.098	16.7	0.097	16.6	0.08	13.0	0.183	31.2	0.035	6.0	0	0	0.001	0.2	0.015	2.6	0.005	0.9	0.025	4.3

815 \*Values from Latasa et al. 2004, †Values from Lopes dos Santos et al. 2016.

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825 Supplementary Table 5. Pigments analyzed in this study. LOD, limit of detection.

Pigment	Abbreviation	Retention time (min)	Detection adsorption wavelength (nm)	LOD (ng/inj)	LOD in 1 L filtered (mg.m-3)
Mg-24-divinyl pheoporphyrin <i>a</i> 5 monomethyl ester	MgDVP	6.3	450	0.015	0.0004
Chlorophyllide <i>a</i> and Chlorophyllide <i>a</i> -lik	Chl <i>a</i> + Chl	6.3	667	0.016	0.0004
Uriolide	Uri	11.6	450	ND	ND
Neoxanthin	Neo	13.5	450	0.008	0.0002
Prasinoxanthin	Pra	14.0	450	0.008	0.0002
Violaxanthin	Vio	14.3	450	0.010	0.0002
Micromonal	MmnaI	15.0	450	ND	ND
Antheraxanthin	Ant	16.3	450	0.012	0.0004
Unknown carotenoid $\lambda_{max}$ 412, 436, 46	Unk 1	16.5	450	ND	ND
Unknown carotenoid $\lambda_{max}$ 452 nm	Unk 2	18.1	450	ND	ND
Lutein	Lut	18.3	450	0.012	0.0002
Dihydrolutein	Dihy	18.6	450	ND	ND
Chlorophyll <i>b</i> -degradation product	Chl <i>b</i> -deg	22	450	ND	ND
Chlorophyll <i>b</i>	Chl <i>b</i>	22.4	450	0.004	0.0001
Chlorophyll <i>b</i> -like	Chl <i>b-like</i>	22.8	450	0.004	0.0001
Chlorophyll <i>a</i> , allomers and epimers	Chl <i>a</i>	24.3	667	0.011	0.0002
Phaeophytin <i>a</i> and Phaeophytin <i>a</i> -like	Phytn <i>a</i> + Ph	25.9	667	0.006	0.0002
$\alpha$ -Carotene and $\beta$ -Carotene	$\alpha$ -car + $\beta$ -car	26.9	450	0.012	0.0004

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