

Mamiellophyceae shift in seasonal predominance in the Baltic Sea

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Running page head: Majaneva et al.: Mamiellophyceae succession

ABSTRACT: The green algae Mamiellophyceae are a group of marine picoeukaryotes. We studied the succession of Mamiellophyceae in the Baltic Sea water column and ice from autumn to summer, using the hypervariable V4 region of the 18S ribosomal RNA gene. We show that species of Mamiellophyceae shift in seasonal predominance and that different species characterize sea ice, under-ice water and the water column in the Baltic Sea.

KEY WORDS: 18S rRNA gene · Hypervariable V4 region · Green algae · Picoeukaryotes · Succession

1. Introduction

Mamiellophyceae, a class of green algae, includes 3 described orders: Mamiellales, Dolichomastigales and Monomastigales (Marin & Melkonian 2010). Dolichomastigales and Monomastigales host many lineages, but they are not abundant in marine waters (Monier et al. 2016). In contrast, the most common Mamiellophyceae in coastal waters—species from the Mamiellales genera *Ostreococcus*, *Bathycoccus* and *Micromonas*—may contribute significantly to the primary production of picoeukaryotes (Worden et al. 2004, Tragin & Vaultot 2018). This has raised research interest in Mamiellophyceae recently, and with the help of molecular methods, the contributions and seasonal patterns of different lineages have become possible to investigate in more detail (e.g. Foulon et al. 2008, Demir-Hilton et al. 2011).

Mamiellophyceae as a class does not show preferences to environmental conditions globally (Tragin & Vaultot 2018), and Mamiellophyceae biogeography is driven largely by geographical location rather than water depth (Monier et al. 2016). For example, species of *Ostreococcus* are globally distributed but are not always found together with usually co-occurring *Bathycoccus* and *Micromonas* (Demir-Hilton et al. 2011), and *Ostreococcus* species are absent in Arctic waters (Tragin & Vaultot 2019). *Ostreococcus* is, however, present in adjacent seasonally sea ice covered areas, for example the Baltic Sea and the White Sea (Majaneva et al. 2012, Belevich et al. 2018).

At the species and genetic strain level, Mamiellophyceae occupy different ecological niches. For example, within the morphospecies *Micromonas pusilla*, different genetic lineages recently divided into several species (Simon et al. 2017) show shifts in abundance along local and basin-wide environmental gradients (Foulon et al. 2008). Similarly, *Ostreococcus* strains show differences in distribution, and co-occurrence at the same geographical location is rare

43 (Demir-Hilton et al. 2011). Metagenomes of the genetically less variable *Bathycoccus*
44 *prasinus* indicate the same (Vannier et al. 2016).

45 In the Baltic Sea, species of *Monomastix*, *Dolichomastix*, *Mamiella*, *Mantoniella* and
46 *Micromonas* are reported (Hällfors 2004, Majaneva et al. 2012). *Mantoniella* sp. appears
47 characteristic for ice, while *B. prasinus* is characteristic for under-ice water (Majaneva et al.
48 2017). However, there are no studies on seasonal patterns or environmental preferences of
49 Mamiellophyceae in the Baltic Sea. Here, we take advantage of our bi-weekly sampling of
50 cold water season protists and describe Mamiellophyceae succession based on V4 reads of the
51 18S ribosomal RNA (18S rRNA) gene in Baltic Sea water and sea ice during the period from
52 autumn to summer.

53 2. Materials and Methods

54 Samples were collected from 2 coastal brackish water (salinity 3.5–6) sites in the
55 northwestern Gulf of Finland, Baltic Sea. Sampling was carried out from 8 October 2012 to 8
56 May 2013, with extra samples on 3 September 2012 and 1 July 2013. The sampling protocol,
57 DNA extraction, PCR setup and sequencing are described in Enberg et al. (2018) and in
58 Supplement 1 at www.int-res.com/articles/suppl/mXXXpXXX_supp/. Enberg et al. (2018)
59 outlined the eukaryotic communities using microscopy and a 310 bp long fragment of the V4
60 region of the 18S rRNA gene (hereafter short V4). Here, we focus on Mamiellophyceae short
61 V4 reads and a longer fragment of the V4 region that was amplified using 574*F and 1132R
62 (Hugerth et al. 2014) primers (hereafter long V4). This long V4 was approximately 540 bp
63 long. The raw reads are available at the ENA SRA repository with the study names
64 PRJEB21047, PRJEB23628 and PRJEB25089.

65 The paired-end sequenced short and long V4 reads were merged using the -
66 `fastq_mergepairs` command in `vsearch v.2.6.2` (Rognes et al. 2016). The quality filtering using
67 the `-fastq_filter` command discarded reads shorter than 320 bases (short V4) and 550 bases
68 (long V4), reads longer than 380 bases (short V4) and 610 bases (long V4), reads with
69 ambiguous bases and reads with over 1 maximum expected error. Primer sequences were
70 removed using the command `trim.seqs` in `mothur v.1.39.5` (Schloss et al. 2009). Exact
71 duplicates were removed using the command `-derep_fulllength` in `vsearch`. Chimeric reads
72 were searched using the command `-uchime_denovo` in `vsearch`. A read was treated as
73 chimeric and removed if its abundance was 5 times lower than its assumed parental reads.
74 Operational taxonomic units (OTUs) were searched using the option `fastidious` in `swarm`
75 `v.2.1.6` (Mahé et al. 2015). The abundance of each OTU in each sample was searched using
76 the `-usearch_global` command (`-id 0.0`) in `vsearch`.

77 The OTUs were assigned taxonomically searching all OTUs first against the NCBI
78 non-redundant nucleotide database (short V4: 9 February 2018, long V4: 10 February 2018)
79 and second against the SILVA_132_SSURef database (Quast et al. 2013), using BLAST
80 `v.2.6.0+` (Zhang et al. 2000). All OTUs affiliated with Mamiellophyceae were selected for the
81 subsequent analyses. Read abundance was normalized to the total number of
82 Mamiellophyceae reads in each sample.

83 To place the OTUs phylogenetically, we took all available sequences of described
84 Mamiellophyceae species together with some relevant uncultured sequences (e.g.
85 Mamiellophyceae DSGM-81) and 6 Pyrenomonadaceae sequences as an outgroup from the
86 SILVA and NCBI Nucleotide databases (23 January 2019). The datasets were combined and
87 aligned with the MAFFT online service (Kato et al. 2017), and the alignment was cut to the
88 length of the long V4. Bayesian phylogenetic analysis was performed with MrBayes `v.3.2.6`
89 (Ronquist et al. 2012). Two independent runs with 4 Markov chains and 1500000 generations

90 were carried out. We did not choose the model prior to the analysis but sampled across the
91 **general time-reversible** model space with gamma-distributed rate variation across sites and a
92 proportion of invariable sites. The resulting estimates (e.g. tree topology) were posterior
93 probability weighted averages of the models. The scripts for methods are provided in
94 Supplement 2. The reads and read abundance are provided in Table S1 in Supplement 3.

95 **3. Results and Discussion**

96 In the dataset, 59 short and 46 long V4 Mamiellophyceae OTUs were present, and
97 after manually checking the alignment and combining identical short and long V4 OTUs, 59
98 OTUs were used in the analyses. Mamiellophyceae OTU richness was significantly lower in
99 sea ice than in under-ice water and the water column (Kruskal-Wallis $\chi^2 = 13.7$, $p = 0.003$,
100 followed by pairwise comparisons using the Mann-Whitney *U*-test). Mamiellophyceae
101 contributed 3.3% of the total abundance of taxonomically assigned OTUs, and they were
102 present in all samples. The lowest Mamiellophyceae read abundance was in the Krogarviken
103 April ice sample (<0.1% of total abundance), and the highest contribution was in the
104 Storfjärden March bottom water sample (17% of total abundance). Mamiellophyceae read
105 abundance was significantly lower in sea ice and under-ice water than in the water column
106 (Kruskal-Wallis $\chi^2 = 26.3$, $p < 0.001$, followed by pairwise comparisons using the Mann-
107 Whitney *U*-test).

108 The Bayesian phylogenetic tree (**Fig. 1**) resembled the latest phylogenies of
109 Mamiellophyceae (Marin & Melkonian 2010, Monier et al. 2016, Simon et al. 2017, **Yau et**
110 **al. unpubl.; preprint doi:10.1101/506915**), and the OTUs represented all 3 described orders
111 with 35 Mamiellales OTUs, 23 Dolichomastigales OTUs and 1 Monomastigales OTU (**Fig.**
112 **1**). OTUs **that referred to** the genus *Mamiella* were not found. In accordance with an earlier
113 global study (Monier et al. 2016), Dolichomastigales were diverse but rare in the Baltic Sea,
114 and the abundant OTUs (>100 **reads in total**, 22 OTUs) were mainly from Mamiellales. The
115 abundant OTUs were associated with *Bathycoccus* (**86923** reads in total, 3 OTUs),
116 *Ostreococcus* (**59313** reads, 3 OTUs), *Micromonas* (**36640** reads, 4 OTUs) and *Mantoniella*
117 (**25982** reads, 7 OTUs). The rest of the abundant OTUs were affiliated with DSGM-81
118 Mamiellophyceae (1147 reads, 3 OTUs) and with *Crustomastix* (362 reads, 2 OTUs) from
119 Dolichomastigales.

120 Different Mamiellophyceae OTUs characterized sea ice, under-ice water and the water
121 column (generalized discriminant analysis of **4 first principal coordinates analysis axes**,
122 analysis based on $\ln[x + 1]$ transformed Bray-Curtis dissimilarities, squared correlations 0.80
123 and 0.58, $p < 0.001$, misclassification error 43.9%).

124 The Mamiellophyceae OTU associated with an uncultured Baltic Sea ice clone
125 FN690723 characterized sea ice (**Fig. 2a,d**, Otu07). This species is clearly sea ice associated,
126 and it is present in the sea ice of the Gulf of Bothnia and the Gulf of Finland in the Baltic Sea
127 (Majaneva et al. 2012) and in the White Sea (Belevich et al. 2018). Otu07 and the uncultured
128 Baltic Sea ice clones form a clade of their own, basal to the *Mantoniella* and *Micromonas*
129 clade in our Bayesian phylogenetic tree (**Fig. 1**) but which are classified as *Mantoniella* in
130 **Tragin & Vaultot (2019)**. *Mantoniella squamata* (Otu14) was the most abundant *Mantoniella*
131 species in the water column in September and October, as was *M. beaufortii* (Otu04) in
132 November to May and OTUs close to *M. antarctica* (Otu21, Otu22) in July (**Fig. 3c**). *M.*
133 *beaufortii* and several *Crustomastix* OTUs characterized under-ice water. The presence of
134 freshwater *Crustomastix* species in low abundance in under-ice water is an indication of river
135 discharge under ice in the area (Kkaartokallio et al. 2007).

136 The abundant *Bathycoccus*, *Ostreococcus* and *Micromonas* species characterized the
137 water column but shifted in predominance during the sampling season (Fig. 2c,f,g).
138 *Ostreococcus mediterraneus* (Otu02) was the most abundant species in October to December,
139 although *Bathycoccus* and *Micromonas* species had a relatively high abundance as well.
140 Otu01, closely related to *Bathycoccus prasinos*, predominated distinctly in January to April,
141 *Micromonas polaris* (Otu06) in May and *M. commoda* (Otu03) in July (Fig. 2c,f).

142 The 4 species of *Micromonas* alternated in dominance in the water column: the
143 predominant species was *M. pusilla* (Otu09) in October surface water and *M. bravo* (Otu10)
144 in bottom water, *M. polaris* (Otu06) in January to May and *M. commoda* (Otu03) in summer
145 and early autumn water (Fig. 3a,b). These shifts in predominance are in line with the thermal
146 niches of *Micromonas* species (Demory et al. 2019).

147 The 2 abundant *Ostreococcus* OTUs—*O. mediterraneus* (Otu02) and the Otu05 basal
148 to the clade of *O. tauri* and *O. lucimarinus*—co-occurred in autumn, and both OTUs were
149 practically absent after March (Fig. 2). The co-occurrence is in accordance with the earlier
150 findings of co-occurring coastal *Ostreococcus* strains (Demir-Hilton et al. 2011) and the
151 absence of *Ostreococcus* in cold polar waters (Tragin & Vaultot 2019). *B. prasinos* had a
152 similar occurrence in the Baltic Sea, being present and abundant only in the autumn (e.g. Fig.
153 2c). The 2 *Ostreococcus* OTUs and *B. prasinos* appear to be characteristic species of the
154 autumn water community (Enberg et al. 2018).

155 The most abundant OTU in our dataset (Otu01, 1 base difference to clone FN690721
156 from Majaneva et al. 2012) was present in all samples. It is closely related to *B. prasinos* (Fig.
157 1), which is the only described species within the genus currently and whose cultures show
158 identical 18S rRNA gene sequences but differing genomes (Vannier et al. 2016, Tragin &
159 Vaultot 2019). It is beyond this note, but the uncorrected interspecific 18S rRNA sequence
160 divergence within the other Mamiellales genera is 1.1 to 5.6%, and the 1.1% uncorrected
161 interspecific divergence of Otu01 and *B. prasinos* implies that Otu01 could be an uncultured,
162 undescribed species of *Bathycoccus*.

163 Here, we have shown that species of Mamiellophyceae shift in seasonal predominance
164 in a coastal brackish water locality in the Baltic Sea and that different species characterize sea
165 ice, under-ice water and the water column during the sea ice covered season. Our findings
166 support the earlier research showing geographic niche partition within Mamiellophyceae
167 (Foulon et al. 2008, Demir-Hilton et al. 2011, Monier et al. 2016) and show that the niche
168 partition holds also for populations in the water and sea ice of the Baltic Sea.

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172 LITERATURE CITED

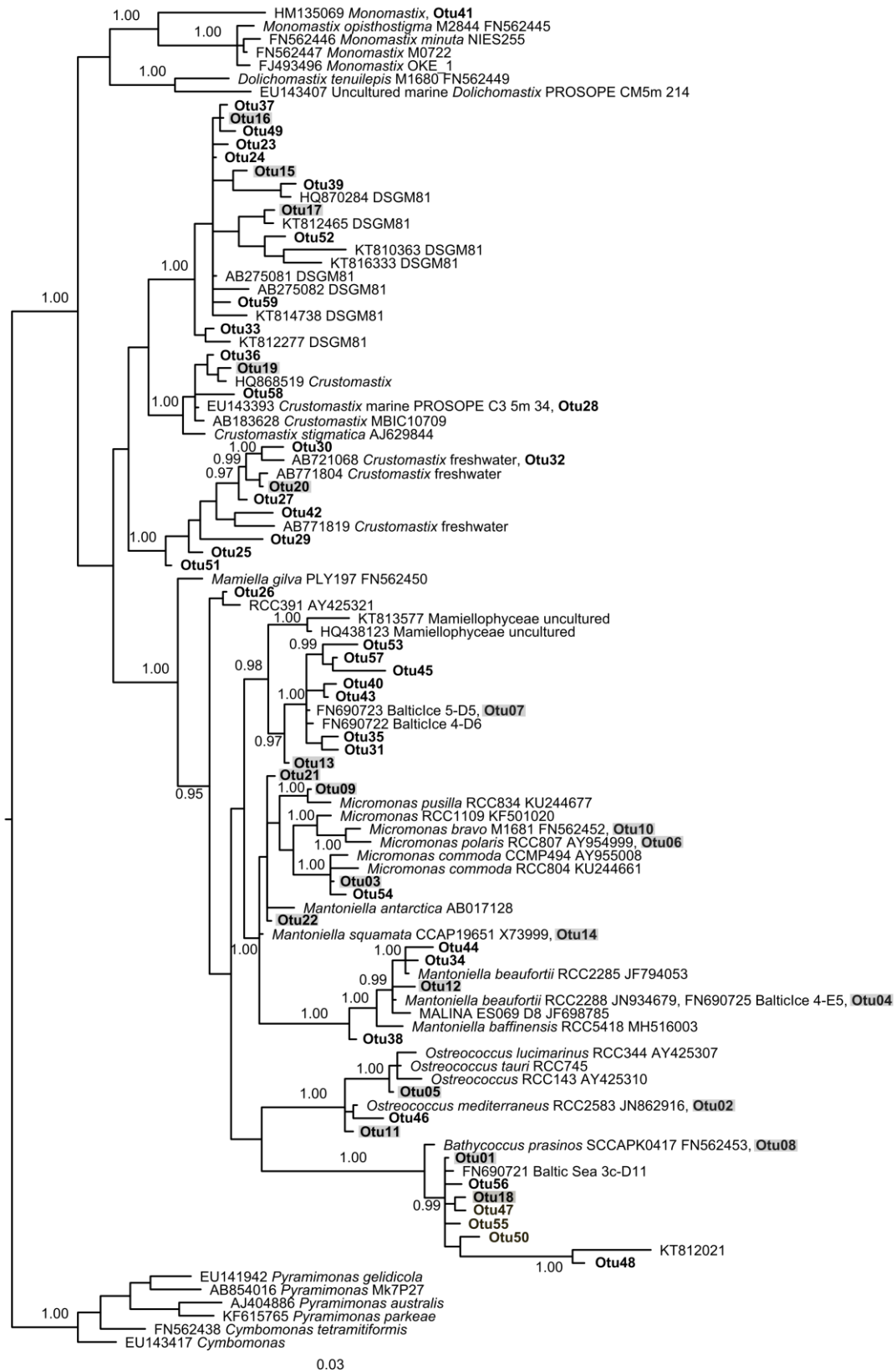
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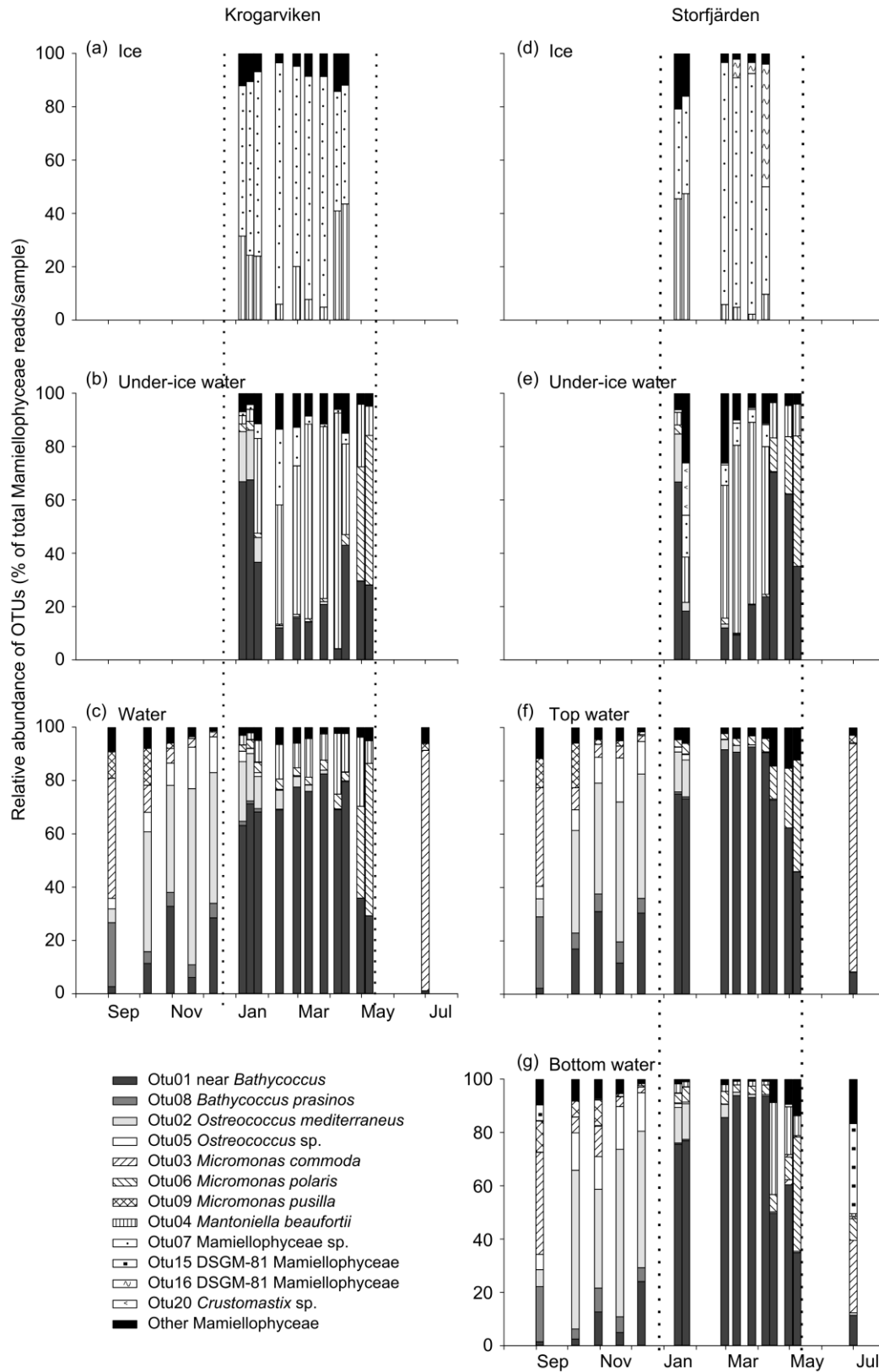
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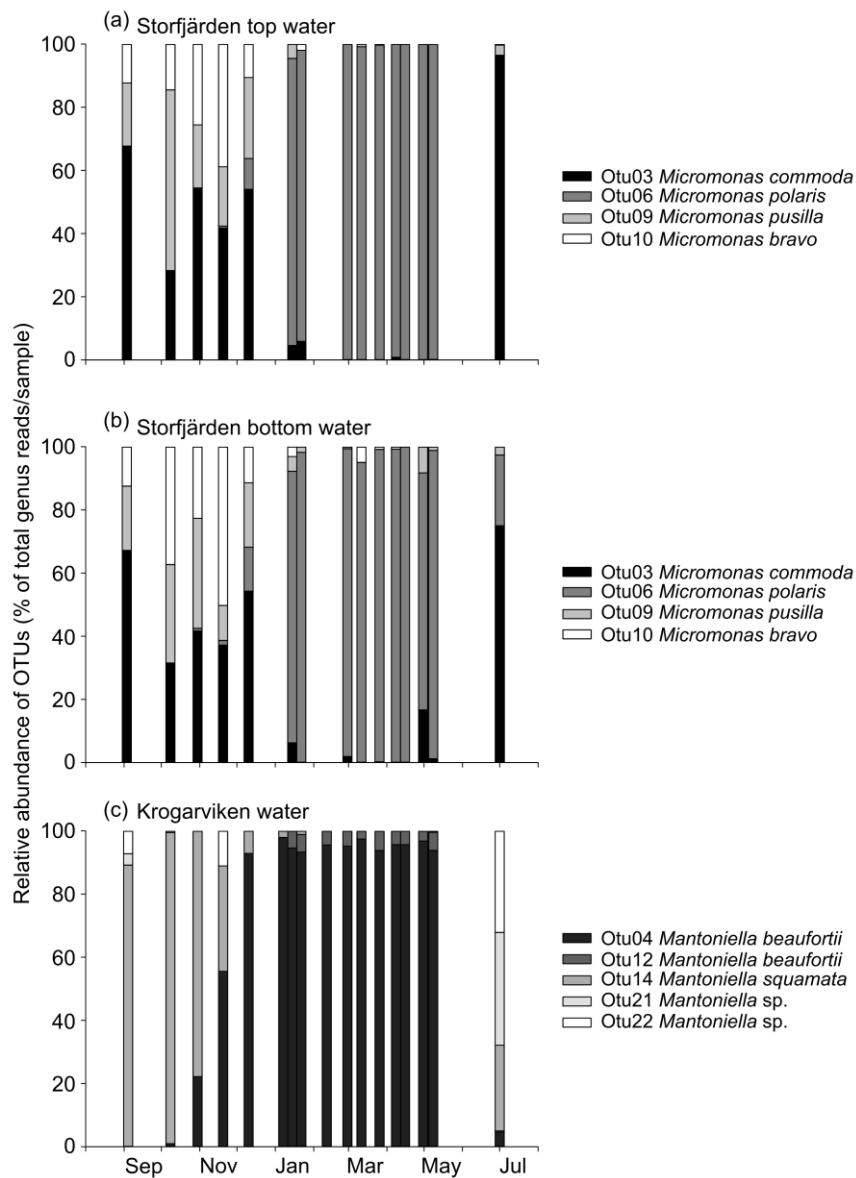
250

251 Fig. 1. Bayesian phylogenetic tree of Mamiellophyceae. Posterior probabilities >0.95 are
 252 shown on the branches. The operational taxonomic units (OTUs) of the current research are
 253 named Otu01, Otu02, etc., in decreasing order by their abundance in the total dataset, and are
 254 in **bold**. OTUs with >100 reads **in total** have a grey background



255

256 Fig. 2. Relative read abundance of the most abundant Mamiellophyceae operational
 257 taxonomic units in sea ice, under-ice water and in the water column at Krogarviken and
 258 Storvfjärden throughout the sampling season. The water depth was 3 m at Krogarviken and ca.
 259 30 m at Storvfjärden. The top water was 0 to 15 m and the bottom water 15 to 30 m at
 260 Storvfjärden. The time between dotted lines represents the ice-covered season



261

262 Fig. 3. Relative read abundance of *Micromonas* and *Mantoniella* operational taxonomic units
 263 in the water column