

<sup>1</sup> TAXONOMIC REASSIGNMENT OF  
<sup>2</sup> *PSEUDOHAPTOLINA BIRGERI* comb. nov.  
<sup>3</sup> (HAPTOPHYTA)<sup>1</sup>

<sup>4</sup> Catherine Gérikas Ribeiro<sup>1,2\*</sup>, Adriana Lopes dos Santos<sup>3,2</sup>, Ian Probert<sup>4</sup>, Daniel  
<sup>5</sup> Vaulot<sup>1,3</sup>, Bente Edvardsen<sup>5</sup>

<sup>6</sup> <sup>1</sup>Sorbonne Université, CNRS, UMR7144, Team ECOMAP, Station Biologique de Roscoff,  
<sup>7</sup> Roscoff, 29680, France

<sup>8</sup> <sup>2</sup>GEMA Center for Genomics, Ecology & Environment, Universidad Mayor, Camino La  
<sup>9</sup> Pirámide, 5750, Huechuraba, Santiago, 8580745, Chile

<sup>10</sup> <sup>3</sup>Asian School of the Environment, Nanyang Technological University, 50 Nanyang Avenue,  
<sup>11</sup> Singapore, 639798, Singapore.

<sup>12</sup> <sup>4</sup>Sorbonne Université, CNRS, FR2424, Roscoff Culture Collection, Station Biologique de  
<sup>13</sup> Roscoff, Roscoff, 29680, France

<sup>14</sup> <sup>5</sup>University of Oslo, Department of Biosciences, Section for Aquatic Biology and Toxicology,  
<sup>15</sup> P.O.Box 1066 Blindern, NO-0316 Oslo, Norway

<sup>16</sup> \*catherine.gerikas@gmail.com

<sup>17</sup> Submitted to: Journal of Phycology

<sup>18</sup> <sup>1</sup> Date: May 1, 2020

19 **Abstract**

20 The haptophyte genus *Pseudohaptolina* (formerly *Chrysochromulina* clade  
21 B1-3) currently harbors two species: *Pseudohaptolina arctica* and *Pseudohap-*  
22 *tolina sorokinii*. In addition, *Chrysochromulina birgeri* is expected to belong  
23 to this genus due to its morphological similarity to *P. sorokinii*, but has not  
24 yet been genetically characterized. A strain belonging to *Pseudohaptolina* was  
25 brought into culture from Arctic waters, characterized by 18S and 28S rRNA  
26 gene sequencing as well as optical and transmission electron microscopy, and de-  
27 posited in the Roscoff Culture Collection with the code RCC5270. Molecular and  
28 morphological data from RCC5270 were compared with those from previously  
29 described *Pseudohaptolina* and *Pseudohaptolina*-like species. Strain RCC5270  
30 showed strong phylogenetic affinity to *P. sorokinii*, but TEM observations showed  
31 that RCC5270 possesses three types of organic body scale, rather than two as orig-  
32 inally described in *P. sorokinii*. We found that the occurrence of three scale types  
33 is likely to have been overlooked in the original descriptions of both *P. sorokinii*  
34 and *C. birgeri*. We also found that environmental metabarcodes identical to the  
35 sequence of RCC5270 were abundant in the location from which *C. birgeri* was  
36 initially described (Gulf of Finland). We conclude that *P. sorokinii* and *C. birgeri*  
37 are conspecific and *P. sorokinii* is therefore synonymous with *C. birgeri*. Based on  
38 its phylogenetic placement and nomenclatural priority we propose the new com-  
39 bination *Pseudohaptolina birgeri* and emend the description of this species.

40 **Introduction**

41 Haptophyte identification is based on both molecular phylogeny and comparison  
42 of morphological features such as cell shape, length and movement of the hap-  
43 tonema, and ornamentation of organic body scales. The genus *Pseudohaptolina*  
44 was erected from the former *Chrysochromulina* B1-3 clade (Edvardsen et al.,  
45 2011). Like most haptophytes, *Pseudohaptolina* are solitary, flagellated and pho-  
46 tosynthetic, with two species currently described: the type species *Pseudohap-*  
47 *tolina arctica* Edvardsen & Eikrem (Edvardsen et al., 2011) and *Pseudohaptolina*  
48 *sorokinii* Stonik, Efimova & Orlova (Orlova et al., 2016). Both of these *Pseu-*  
49 *dohaptolina* species were described from high latitude northern hemisphere ma-  
50 rine waters, *P. sorokinii* having been collected during an under-ice algal bloom in  
51 Amurskiy Bay in the northwestern Sea of Japan (Orlova et al., 2016). A new rep-  
52 resentative strain from the genus *Pseudohaptolina* was brought into culture from  
53 Canadian Arctic waters in 2016 (Gérikas Ribeiro et al., 2020) allowing compar-  
54 ison to previously described *Pseudohaptolina* species using morphological and  
55 genetic features.

56 **Material and Methods**

57 Strain RCC5270 was isolated into clonal culture from Canadian Arctic waters in  
58 2016 (Gérikas Ribeiro et al., 2020), more specifically from Baffin Bay close to the  
59 Inuit village of Qikiqtarjuaq, Nunavut on Baffin Island (67°28' N, 63°47' W). The  
60 strain was identified by 18S rRNA gene sequencing and optical microscopy and  
61 deposited in the Roscoff Culture Collection (<http://roscoff-culture-collection.org>)  
62 with the code RCC5270. Strain RCC5268 was recovered from the same sample  
63 than RCC5270 and its 18S rRNA sequence (MH764749) shares 100% similarity

64 of with that of RCC5270.

65 The nearly complete 18S rRNA gene was amplified using the  
66 primers 63F (5'-ACGCTTGTCTCAAAGATTA-3') and 1818R (5'-  
67 ACGGAAACCTTGTACGA-3') (Lepère et al., 2011) and sequenced using the  
68 same primers and the internal primer 528F (5'-CCGCGGTAATTCCAGCTC-  
69 3') (Zhu et al., 2005). The 28S rRNA gene was amplified and sequenced  
70 using primers D1R (5'-ACCCGCTGAATTAAAGCATA-3') and D3Ca (5'-  
71 ACGAACGATTGCACGTCAG-3') (Lenaers et al., 1989). Sequencing was  
72 performed at Macrogen Europe (<https://dna.macrogen-europe.com>). Consensus  
73 sequences were generated using *de novo* assembly in Geneious® 10 (Kearse  
74 et al., 2012). The RCC5270 18S and 28S rRNA gene sequences were deposited  
75 in GenBank under accession numbers MT311519 and MT311520, respectively.  
76 For phylogenies, sequences from strain RCC5270 were aligned to closely related  
77 Haptophyta sequences from Genbank using the Muscle plugin in Geneious® 10  
78 (Kearse et al., 2012).

79 Samples for transmission electron microscopy (TEM) were prepared as  
80 whole mounts fixed with osmium vapor following Eikrem (1996) with slight  
81 modifications (cooling of all equipment). Observations were made using a  
82 Jeol JEM-2010 FEG at the Imaging Core Facility at the Station Biologique  
83 de Roscoff, France. The size of more than 100 scales from RCC5270 and  
84 RCC5268 was measured from TEM micrographs using the imaging soft-  
85 ware ImageJ (<https://imagej.nih.gov/ij/>). Representative images are available at  
86 <http://www.roscoff-culture-collection.org/rcc-strain-details/5270>.

87 In order to determine the oceanic distribution of the species correspond-

88 ing to RCC5270, we examined a large set of publicly available metabar-  
89 code datasets (Table 1) covering the V4 and V9 region of the 18S rRNA  
90 gene. Twenty-one oceanic 18S rRNA metabarcode datasets were downloaded  
91 and reprocessed with the *dada2* R package (Callahan et al., 2016) following  
92 the standard operating procedure <https://benjneb.github.io/dada2/tutorial.html>  
93 in order to produce amplicon single variants (ASVs). The taxonomy of  
94 each ASV was assigned using the *dada2* assignTaxonomy function against  
95 version 4.12 of the PR<sup>2</sup> database (Guillou et al., 2013) available at  
96 <https://github.com/pr2database/pr2database/releases/tag/v4.12.0>. Twenty datasets  
97 corresponded to the V4 of the 18S rRNA gene, and one to the V9 region (Tara  
98 *Oceans*). ASVs with a 100% match to the sequence of RCC5270 were selected  
99 and the number of reads in each sample determined using the R library dplyr.  
100 Maps and figures were drawn using the R libraries *ggplot2*, *sf* and *cowplot*.

## 101 Results and Discussion

102 The 18S rRNA gene sequence from RCC5270 was compared with similar se-  
103 quences in GenBank including those from previously described *Pseudohaptolina*  
104 species. The best match of the sequence was to the two *P. sorokinii* 18S rRNA  
105 sequences in GenBank (KF684962 and KU589286), both linked to its original  
106 description, although only KF684962 is cited in the text of the original descrip-  
107 tion. The 18S rRNA gene sequence of strain RCC5270 differs from sequence  
108 KF684962 by five base pairs (four substitutions and one deletion) in a 1,655 bp  
109 alignment and by only one base pair deletion when compared to KU589286 (1,213  
110 bp alignment). The divergences from KF684962 seem to originate from sequenc-  
111 ing errors in the *P. sorokinii* description, since they occur in well conserved posi-

112 tions (Figure 1) and when there is a base variation within these positions in related  
113 haptophytes, they do not match with those in the *P. sorokinii* sequence (Figure 1).  
114 Furthermore, the two sequences linked to the original description of *P. sorokinii*  
115 do not share the same substitutions.

116 The 28S rRNA gene sequence from RCC5270 has a six base pair difference  
117 to the only *P. sorokinii* 28S rRNA sequence available in GenBank (KU589284),  
118 which did not originate from the same isolate used for the description of *P.*  
119 *sorokinii*, and is not mentioned in Orlova et al. (2016). Both RCC5270 28S  
120 rRNA and KU589284 best hits in GenBank correspond to the environmental clone  
121 KU898784 from a sea ice sample in the Barrow Sea (Hassett et al., 2017), with  
122 100% and 98% similarity, respectively.

123 The shape, size and ornamentation of the organic body scales are taxonomi-  
124 cally important characters in Haptophyta, and usually more than one type of body  
125 scale occurs per species. *Chrysochromulina birgeri* Hällfors & Niemi (Hällfors  
126 and Niemi, 1974) was described before the genus *Pseudohaptolina* was erected,  
127 but is expected to be incorporated within *Pseudohaptolina* based on its morpho-  
128 logical similarity to members of this genus. The discrimination between *C. birgeri*  
129 and other *Pseudohaptolina* species is only possible through morphological exam-  
130 ination, since no molecular data or culture strains are available from its first de-  
131 scription (Hällfors 1974). *C. birgeri*, *P. arctica* and *P. sorokinii* were all described  
132 as possessing two types of body scale (Hällfors and Niemi, 1974; Edvardsen et  
133 al., 2011; Orlova et al., 2016), usually referred to as ‘small’ and ‘large’ scales.  
134 For the *P. sorokinii* description (Orlova et al., 2016), three morphological fea-  
135 tures of the organic body scales are indicated as distinctive enough to assign it

136 to a new species: horn morphology, shape of the connecting bridge and density  
137 of radial ribs. However, apart from the feature ‘number of radial ribs arranged in  
138 quadrants’ present in the so-called small scales, all other measurements overlap  
139 to some extent with those recorded for *C. birgeri* (see Table 1 from Orlova et al.,  
140 2016, page 511).

141 In general, the scale morphology of RCC5270 corresponds closely to that de-  
142 scribed for *C. birgeri* and *P. sorokinii*, including a radial pattern of ribs arranged  
143 in quadrants that coincide with the two orthogonal axes of the scale, and two  
144 horn-like projections connected by a straight or slightly curved bridge (Figure 2).  
145 However, both morphometric data and observations of TEM images of RCC5270  
146 indicate that at least three types of organic scales can be differentiated (Table 2,  
147 Figure 2) using scale length, width and distance between the horns, and number  
148 of radial ribs per quadrant (Figure 4). Small scales of strain RCC5270 have 37-39  
149 ribs on each quadrant (Figure 2B), as in the description of *C. birgeri* (Hällfors &  
150 Niemi, 1974), whereas the medium scales have 54-56 and large scales have 63-  
151 68 radial ribs per quadrant (Table 2). The distinction between small and medium  
152 scales is, however, most readily visible when comparing scale length *versus* width  
153 (Figure 4A). Medium and large scales have somewhat overlapping sizes, so their  
154 separation is better achieved by comparing distance between the horn bases *ver-*  
155 *sus* width (Figure 4B), due to a clear distinctive horn bridge structure, with large  
156 scales presenting bigger and usually slightly curved bridges (Figure 2).

157 When measurements are conducted on the images displayed in the original  
158 descriptions, we found that the three types of scales can be distinguished for *P.*  
159 *sorokinii* (Figure 3A, Figure 4) and most likely also for *C. birgeri*, as shown in

160 Figure 3D. Two *P. sorokinii* organic scales, identified as ‘small scales’ in the orig-  
161 inal description (Figure 3B and C, see also Orlova et al., 2016, page 510, figures  
162 9 and 11), fall in the same size range as the ‘medium’ scales identified here (Fig-  
163 ure 4), which impacts the number of ribs counted. In addition, independent mea-  
164 surements of small scales depicted in figure 8 of the original paper (Figure 3A in  
165 the present work), which are true small scales, fall outside the size range of small  
166 scales described by Orlova et al. (2016) (Figure 4). Unfortunately, the resolution  
167 of available *P. sorokinii* images is not sufficient to perform an independent count  
168 of the ribs in the small scales. The size of the connecting bridge was used by  
169 Orlova et al. (2016) as a distinctive feature of large scales, so small and medium  
170 scales were probably grouped together, which might have led to the discrepan-  
171 cies observed in the number of ribs per quadrant reported in the *P. sorokinii* de-  
172 scription. In contrast, in the *C. birgeri* description medium and large scales with  
173 evident differences in the connecting bridge structure were grouped together as  
174 ‘large’ (Figure 3E and F). It is noteworthy that neither *P. sorokinii* nor RCC5270  
175 scale measurements correspond precisely to the size limits described for *C. birgeri*  
176 (Hällfors & Niemi, 1974), particularly for small scales (Figure 4).

177 Other morphological characteristics used to differentiate *P. sorokinii* from *C.*  
178 *birgeri* by Orlova et al. (2016) are horn length and the shape of the connecting  
179 bridge. Orlova et al. (2016) reported long horn projections and curved connecting  
180 bridges, in contrast to the description of *C. birgeri*, although long horn-like projec-  
181 tions connected by a curved bridge in large scales have already been reported for  
182 *C. birgeri* (Takahashi, 1981; Hällfors and Thomsen, 1979). The horn projections  
183 of large scales of RCC5270 are in general smaller than observed by Orlova et al.

184 (2016), but are somewhat superimposed within their size range (Table 2). We also  
185 observed curved connecting bridges in the large scales (Figure 2A). There is there-  
186 fore considerable overlap but some variability in the size and features of scales of  
187 RCC5270, *P. sorokinii* and *C. birgeri* which might reflect morphological plasticity  
188 within a single species, since heteromorphic life cycles have been observed within  
189 the Prymnesiales (Paasche et al., 1990; Edvardsen and Vaulot, 1996).

190 The metabarcode datasets used to determine the oceanic distribution of  
191 RCC5270 correspond to more than 2,200 samples included in large scale sur-  
192 veys such as Ocean Sampling Day (OSD) and the Tara *Oceans* and Malaspina  
193 expeditions that sampled a wide range of coastal and oceanic waters as well as  
194 more limited studies from polar waters and the Baltic Sea. We did not retrieve  
195 any V9 metabarcodes identical to the RCC5270 sequence. We did, however, re-  
196 trieve six V4 metabarcodes (ASVs) that were 100% identical to the RCC5270  
197 sequence (Figure S1). In contrast, no exact match was found to either KF684962  
198 or KU589286 *P. sorokinii* in any of these datasets, which further corroborates  
199 the assumption that the mismatch between 18S rRNA *P. sorokinii* and RCC5270  
200 sequences are due to sequencing errors. The RCC5270 metabarcodes were only  
201 observed in the Arctic Ocean and in the Baltic Sea from ice and water samples  
202 as well from algal aggregates collected from the deep-sea floor (Figure 5A-B).  
203 Metabarcodes identical to the sequence of RCC5270 were particularly abundant  
204 in three datasets (Table 1) from the Polarstern expedition in the Central Arctic  
205 Ocean (Rapp et al., 2018), from the Nares strait, the northernmost outflow gate-  
206 way of Baffin Bay (Kalenitchenko et al., 2019) and from the Gulf of Finland  
207 (Baltic Sea) (Enberg et al., 2018). At the latter location, which corresponds to the

208 region from which *C. birgeri* was initially described, metabarcodes identical to  
209 the RCC5270 sequence first appeared in February in the ice where they peaked in  
210 early March and then increased massively in the water column one month later,  
211 representing up to 70% of the metabarcodes at the time the ice melted in mid-  
212 April (Figure 5C). These data indicate that RCC5270 is an ice alga that can seed  
213 and proliferate in the water column and even accumulate on the deep-sea floor.

214 **Conclusions**

215 We isolated a culture strain from the Arctic which was genetically affiliated to *P.*  
216 *sorokinii*. Morphological data indicate that a third scale type was overlooked in  
217 the original description of *P. sorokinii* (Orlova et al., 2016), impacting the num-  
218 ber of radiating ribs described for each scale type. We also found that *C. birgeri*  
219 cells have three types of organic body scale, not two as reported in the original de-  
220 scription (Hälfors and Niemi, 1974). Metabarcode data indicates that sequences  
221 identical to that of RCC5270 were abundant near the type locality of *C. birgerii*.  
222 We conclude that *P. sorokinii* is conspecific with the formerly described *C. birg-*  
223 *eri* and we therefore transfer *C. birgeri* to the genus *Pseudohaptolina* and emend  
224 its description. *P. birgeri* is the valid name for this species due to nomenclatural  
225 priority over *P. sorokinii*.

226 **Taxonomic appendix**

227 *Pseudohaptolina birgeri* (Hälfors & Niemi) Ribeiro and Edvardsen comb. nov.  
228 emend. Ribeiro and Edvardsen  
229 BASIONYM: *Chrysochromulina birgeri* Hälfors & Niemi in Hälfors &  
230 Niemi (1974). Memoranda Societatis pro Fauna et Flora Fennica 50. Drawing

231 Fig. 4.

232 SYNONYM: *Pseudohaptolina sorokinii* Stonik, Efimova & Orlova.

233 EMENDED DESCRIPTION: Scaly covering composed of three round to oval  
 234 scale types. Small scales have width x length c. 0.6-1.4 x 1.1-1.7, medium scales  
 235 c. 1.1-2 x 1.5-2.4 and large scales c. 1.1-2.1 x 1.9-2.8 nm. All scales with radial  
 236 ribs on both distal and proximal faces. Small scales have 37-39 radial ribs per  
 237 quadrant, medium scales 54-60 and large scales 63-68. Medium and large scales  
 238 have two horns on the distal face. The distance and form of the horns are different  
 239 in medium and large scales.

240 **References**

- 241 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, et al. 2016.  
 242 DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**(7): 581–583. doi:10.1038/nmeth.3869.
- 243 Edvardsen B, Eikrem W, Throndsen J, Sáez AG, Probert I, et al. 2011. Ribosomal DNA phylogenies and a morphological revision provide the basis for a revised taxonomy of the Prymnesiales (Haptophyta). *European Journal of Phycology* **46**(3): 202–228. doi:10.1080/09670262.2011.594095.
- 244 Edvardsen B, Vaulot D. 1996. Ploidy analysis of the two motile forms of *Chrysochromulina polylepis* (Prymnesiophyceae). *Journal of Phycology* **32**: 94–102.
- 245 Eikrem W. 1996. *Chrysochromulina throndsenii* sp. nov. (Prymnesiophyceae). Description of a new haptophyte flagellate from Norwegian waters. *Phycologia* **35**(5): 377–380.
- 246 Enberg S, Majaneva M, Autio R, Blomster J, Rintala JM. 2018. Phases of mi-

- 255 croalgal succession in sea ice and the water column in the Baltic Sea  
256 from autumn to spring. *Marine Ecology Progress Series* **599**: 19–34. doi:  
257 10.3354/meps12645.
- 258 Gérikas Ribeiro C, Lopes dos Santos A, Marie D, Le Gall F, Probert I, et al.  
259 2020. Culturable diversity of Arctic phytoplankton during pack ice melting.  
260 *Elementa: Science of the Anthropocene* **8**: 6. doi:10.1101/642264v1.
- 261 Guillou L, Bachar D, Audic S, Bass D, Berney C, et al. 2013. The Protist Ri-  
262 bosomal Reference database (PR<sup>2</sup>): a catalog of unicellular eukaryote Small  
263 Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Research*  
264 **41**(D1): D597–D604. doi:10.1093/nar/gks1160.
- 265 Hälfors G, Niemi A. 1974. *Chrysochromulina* (Haptophyceae) bloom under the  
266 ice in the Tvarminne Archipelago, southern coast of Finland. *Memoranda*  
267 *Societatis pro Fauna et Flora Fennica* **50**: 89–104.
- 268 Hälfors G, Thomsen HA. 1979. Further observations on Chrysochromulina birg-  
269 eri (Prymnesiophyceae) from the Tvärminne archipelago, SW coast of Fin-  
270 land. *Acta Bot Fennica* **110**(July): 41–46.
- 271 Hassett BT, Ducluzeau ALL, Collins RE, Gradinger R. 2017. Spatial distribution  
272 of aquatic marine fungi across the western Arctic and sub-Arctic. *Environ-  
273 mental Microbiology* **19**(2): 475–484. doi:10.1111/1462-2920.13371.
- 274 Kalenitchenko D, Joli N, Potvin M, Tremblay JÉ, Lovejoy C. 2019. Biodiver-  
275 sity and species change in the Arctic Ocean: A view through the lens of  
276 Nares Strait. *Frontiers in Marine Science* **6**(August): 1–17. doi:10.3389/  
277 fmars.2019.00479.
- 278 Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, et al. 2012. Geneious

- 279 Basic: An integrated and extendable desktop software platform for the orga-  
280 nization and analysis of sequence data. *Bioinformatics* **28**(12): 1647–1649.  
281 doi:10.1093/bioinformatics/bts199.
- 282 Lenaers G, Maroteaux L, Michot B, Herzog M. 1989. Dinoflagellates in evolution.  
283 A molecular phylogenetic analysis of large subunit ribosomal RNA. *Journal  
284 of Molecular Evolution* **29**(1): 40–51.
- 285 Lepère C, Demura M, Kawachi M, Romac S, Probert I, et al. 2011. Whole-  
286 genome amplification (WGA) of marine photosynthetic eukaryote popula-  
287 tions. *FEMS Microbiology Ecology* **76**: 513–523. doi:10.1111/j.1574-6941.  
288 2011.01072.x.
- 289 Orlova TY, Efimova KV, Stonik IV. 2016. Morphology and molecular phylogeny  
290 of *Pseudohaptolina sorokinii* sp . nov . (Prymnesiales, Haptophyta) from the  
291 Sea of Japan , Russia. *Phycologia* **55**(5): 506–514. doi:10.2216/15-107.1.
- 292 Paasche E, Edvardsen B, Eikrem W. 1990. A possible alternate stage in the life  
293 cycle of *Chrysochromulina polylepis* Manton et Parke (Prymnesiophyceae).  
294 *Nova Hedwigia Beiheft* **100**(May 2014): 91–99.
- 295 Rapp JZ, Fernández-Méndez M, Bienhold C, Boetius A. 2018. Effects of ice-algal  
296 aggregate export on the connectivity of bacterial communities in the central  
297 Arctic Ocean. *Frontiers in Microbiology* **9**(May): 1035. doi:10.3389/fmicb.  
298 2018.01035.
- 299 Takahashi E. 1981. Floristic study of ice algae in the sea ice of a lagoon, Lake  
300 Saroma, Hokkaido, Japan. *Memoirs of the National Institute of Polar Re-  
301 search* **34**: 49–56.
- 302 Zhu F, Massana R, Not F, Marie D, Vaulot D. 2005. Mapping of picoeucaryotes

303 in marine ecosystems with quantitative PCR of the 18S rRNA gene. *FEMS*  
304 *Microbiology Ecology* **52**: 79–92. doi:10.1016/j.femsec.2004.10.006.

305 **Contributions**

306 Contributed to conception and design: CGR, IP, DV, BE  
307 Contributed to acquisition of data: CGR, ALS, IP, DV, BE  
308 Contributed to analysis and interpretation of data: CGR, ALS, IP, DV, BE  
309 Drafted and/or revised the article: CGR, ALS, IP, DV, BE  
310 Approved the submitted version for publication: CGR, ALS, IP, DV, BE

311

312 **Acknowledgments**

313 We are grateful to Sophie Le Panse from the Merimage microscopy platform at the  
314 Roscoff Marine Station for assistance with the transmission electron micrographs  
315 and to the Roscoff Culture Collection for maintenance of the algal strain.

316 **Funding information**

317 Financial support for this work was provided by the Green Edge project (ANR-  
318 14-CE01-0017, Fondation Total), the ANR PhytoPol (ANR-15-CE02-0007) and  
319 TaxMArc (Research Council of Norway, 268286/E40). ALS was supported  
320 by FONDECYT grant PiSCOSouth (N1171802). CGR was supported by the  
321 FONDECYT project 3190827.

322 **Competing interests**

323 The authors have no competing interests.

<sup>324</sup> **Data accessibility statement**

<sup>325</sup> Supporting data have been deposited to GitHub <https://github.com/vaulot/Paper->

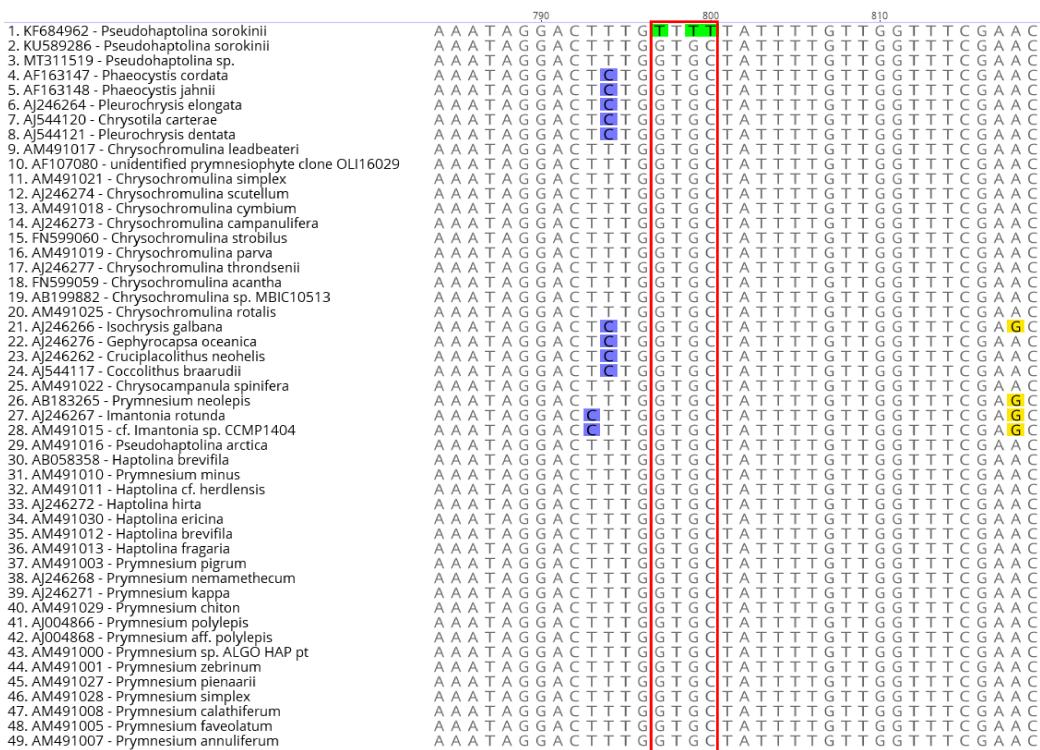
<sup>326</sup> 2020-Ribeiro-Pseudohaptolina.

**Table 1. Datasets considered for metabarcoding analysis. These 21 datasets correspond to the V4 (20) and V9 (1) regions of the 18S rRNA gene. All datasets have been processed with the dada2 software (Callahan et al., 2016) to extract ASV (amplicon single variants) and assigned using the PR2 database (Guillou et al., 2013).**

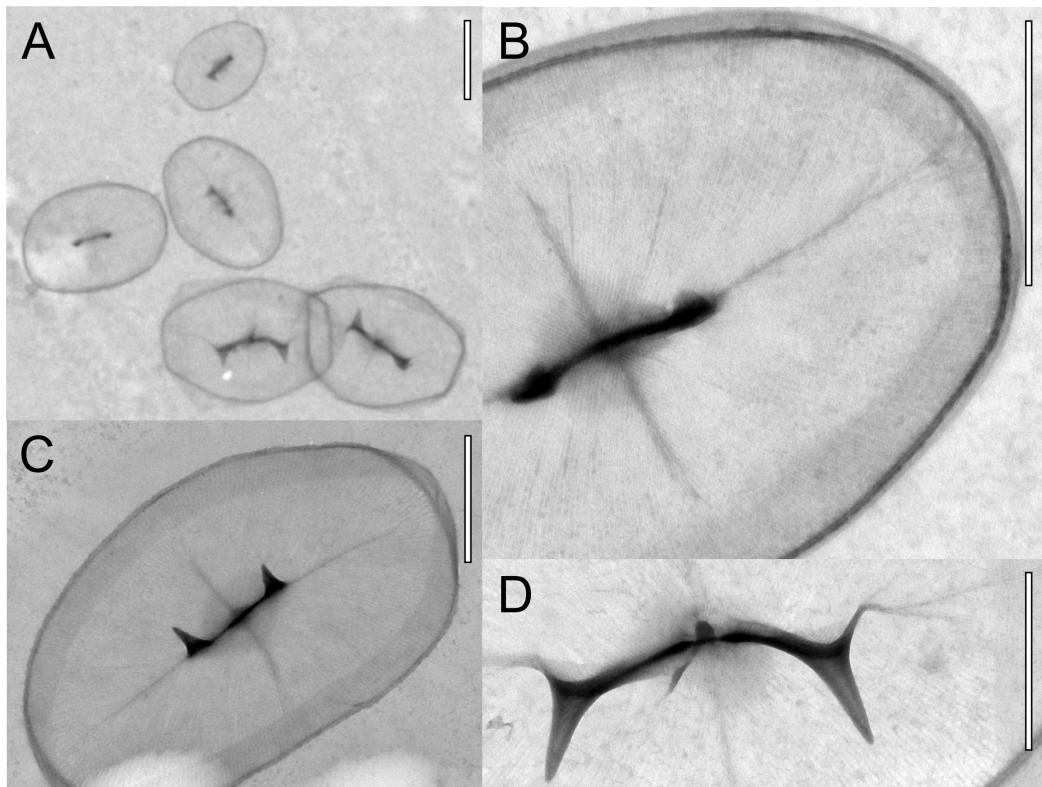
| ID | Gene region | Description                                             | Oceanic region    | BioProject or Repository | DOI paper                                                                | Reads  | Substrate             |
|----|-------------|---------------------------------------------------------|-------------------|--------------------------|--------------------------------------------------------------------------|--------|-----------------------|
| 5  | V4          | Arctic Ocean, Beaufort Sea, MALINA cruise - 2009        | Arctic Ocean      | PRINNA202104             | 10.1080/09670262.2015.1077395                                            | 16     | ice                   |
| 6  | V4          | Central Arctic Ocean - 2012                             | Arctic Ocean      | PRIEB7577                | 10.1371/journal.pone.0148512                                             |        | ice                   |
| 9  | V4          | Nansen Basin - 2012                                     | Arctic Ocean      | PRIEB11449               | 10.1038/e41598-018-27705-6                                               |        |                       |
| 37 | V4          | Baffin Bay - 2013                                       | Arctic Ocean      | PRINNA383398             | 10.1007/s00248-017-1076-x                                                | 62     | ice                   |
| 38 | V4          | White Sea - 2013-2015                                   | Arctic Ocean      | PRINNA368621             | 10.3891/mincb.2018.01035.                                                |        | algae, ice, water     |
| 39 | V4          | Arctic Ocean - Polarstern expedition ARK-XXVII/3 - 2012 | Arctic Ocean      | PRIEB23005               | 10.1128/AEM.02737-14                                                     | 14212  | water                 |
| 40 | V4          | Arctic Ocean Survey - 2005-2011                         | Arctic Ocean      | PRINNA243055             | 10.1128/AEM.02737-14                                                     | 17     | water                 |
| 41 | V4          | Chukchi Sea - ICESCAPE - 2010                           | Arctic Ocean      | PRINNA217438             | 10.1128/AEM.02737-14                                                     |        |                       |
| 42 | V4          | Nares Strait - 2014                                     | Arctic Ocean      | PRIEB24314               | 10.3891/mincb.2019.00479                                                 | 65898  | water                 |
| 20 | V4          | Oslo fjord - 2009-2011                                  | Atlantic Ocean    | PRINNA497792             | 10.1111/jeu.12700                                                        |        |                       |
| 19 | V4          | Gulf of Finland - 2012-2013                             | Baltic Sea        | PRIEB21047               | 10.3534/meps.2645                                                        | 127118 | first year ice, water |
| 43 | V4          | Gdansk Gulf - 2012                                      | Baltic Sea        | PRIEB23971               | 10.1002/lno.11177                                                        |        |                       |
| 36 | V4          | Blanes Time Series - 2004-2013                          | Mediterranean Sea | PRIEB23788               | 10.1111/mec.14929                                                        |        |                       |
| 49 | V4          | Bay of Naples - 2011                                    | Mediterranean Sea | PRIEB24595               | 10.1093/femsed/fw200                                                     |        |                       |
| 1  | V4          | Ocean Sampling Day 2014 V4 LGC                          | Ocean survey      | PRIEB8682                | 10.1186/s13742-015-0066-5                                                |        |                       |
| 2  | V4          | Ocean Sampling Day 2015 V4                              | Ocean survey      |                          | https://github.com/MicroB3-IS/obs-d-analysis/wiki/Guide-to-OSD-2015-data |        |                       |
| 3  | V4          | Ocean Sampling Day 2014 V4 LW                           | Ocean survey      |                          | https://github.com/MicroB3-IS/obs-d-analysis/wiki/Guide-to-OSD-2014-data |        |                       |
| 34 | V4          | Malaspina expedition - vertical profiles - 2010-2011    | Ocean survey      | PRIEB23771               | 10.1038/s41396-019-0506-9                                                |        |                       |
| 35 | V4          | Malaspina expedition - surface - 2010-2011              | Southern Ocean    | PRIEB23913               | 10.1007/s00300-015-1815-8                                                |        |                       |
| 11 | V4          | Fields Bay, Antarctic - 2013                            | Ocean survey      | PRINNA254097             | 10.1126/science.1261605                                                  |        |                       |
| 15 | V9          | Tara Oceans - 2009-2012                                 | Ocean survey      | PRIEB6610                |                                                                          |        |                       |

**Table 2. Comparison of organic scale measurements between RCC5270, *P. sorokinii* original description (Orlova et al., 2016), *P. sorokinii* independent measurements, and *C. birgeri* original description (Hällfors & Niemi, 1974).**

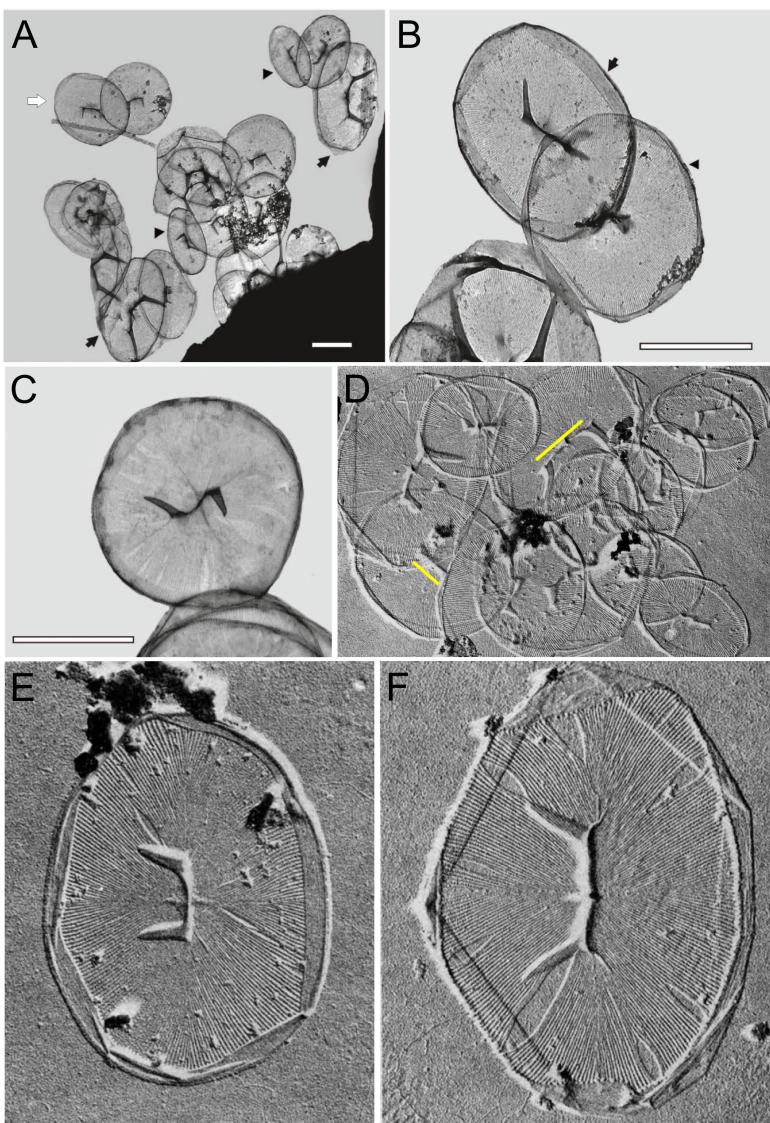
| Measurements                                                  | RCC5270                    | <i>P. sorokinii</i> description | <i>P. sorokinii</i> images | <i>C. birgeri</i> description |
|---------------------------------------------------------------|----------------------------|---------------------------------|----------------------------|-------------------------------|
| <b>Scale length (<math>\mu\text{m}</math>)</b>                |                            |                                 |                            |                               |
| small                                                         | 1.1 - 1.4 (1.2 $\pm$ 0.1)  | 1.6 - 2.0 (1.9 $\pm$ 0.03)      | 1.67 - 1.73                | 1.5 - 1.7                     |
| medium                                                        | 1.5 - 2.4 (1.8 $\pm$ 0.2)  | NA                              | 1.8 - 2                    | NA                            |
| large                                                         | 1.9 - 2.5 (2.2 $\pm$ 0.2)  | 2.1 - 3.2 (2.6 $\pm$ 0.1)       | 2.7 - 2.8                  | 2.2 - 2.6                     |
| <b>Scale width (<math>\mu\text{m}</math>)</b>                 |                            |                                 |                            |                               |
| small                                                         | 0.6 - 1 (0.8 $\pm$ 0.1)    | 1.2 - 1.9 (1.5 $\pm$ 0.05)      | 0.90 - 0.95                | 1.1 - 1.4                     |
| medium                                                        | 1.1 - 1.7 (1.3 $\pm$ 0.2)  | NA                              | 1.3 - 2                    | NA                            |
| large                                                         | 1.1 - 1.8 (1.5 $\pm$ 0.2)  | 1.6 - 2.3 (1.9 $\pm$ 0.1)       | 1.5 - 1.61                 | 1.7 - 2.1                     |
| <b>Distance between horn bases (<math>\mu\text{m}</math>)</b> |                            |                                 |                            |                               |
| small                                                         | 0.2 - 0.4 (0.3 $\pm$ 0.04) | 0.3 - 0.4 (0.4 $\pm$ 0.02)      | 0.34 - 0.37                | 0.3 - 0.4                     |
| medium                                                        | 0.3 - 0.6 (0.4 $\pm$ 0.1)  | NA                              | 0.4 - 0.5                  | NA                            |
| large                                                         | 0.6 - 1.1 (0.7 $\pm$ 0.1)  | 0.5 - 0.9 (0.7 $\pm$ 0.04)      | 1.02 - 1.03                | 0.4 - 0.8                     |
| <b>Horn measurements (<math>\mu\text{m}</math>)</b>           |                            |                                 |                            |                               |
| small                                                         | 0.1 - 0.2 (0.1 $\pm$ 0.1)  | 0.2 - 0.4 (0.3 $\pm$ 0.02)      | 0.26 - 0.3                 | 0.1 - 0.2                     |
| medium                                                        | 0.1 - 0.2 (0.2 $\pm$ 0.03) | NA                              | 0.3 - 0.4                  | NA                            |
| large                                                         | 0.2 - 0.6 (0.3 $\pm$ 0.1)  | 0.5 - 0.9 (0.7 $\pm$ 0.03)      | 0.7 - 0.8                  | 0.2 - 0.6                     |
| <b>Number of ribs per quadrant</b>                            |                            |                                 |                            |                               |
| small                                                         | 37 - 39                    | 49 - 57 (52.2 $\pm$ 0.8)        | NA                         | c. 38                         |
| medium                                                        | 54 - 56                    | NA                              | 54- 60                     | NA                            |
| large                                                         | 63 - 68                    | 52 - 64 (57.8 $\pm$ 1.5)        | NA                         | 55 - 68                       |



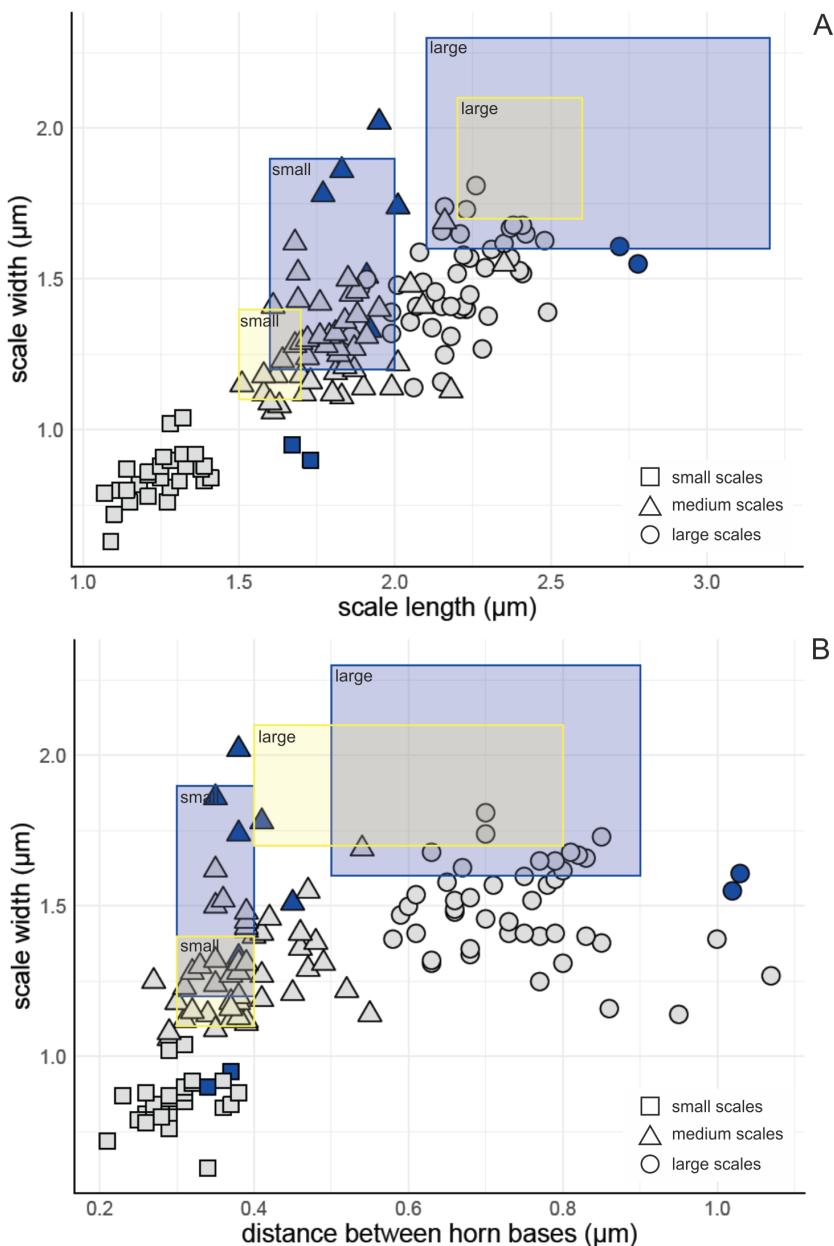
**Figure 1.** Partial V4 18S rRNA gene sequence alignment showing RCC5270 and *P. sorokinii* KF684962 sequence in comparison to closely related groups; three substitutions are visible in the latter, but they are not shared by any other sequence, including *P. sorokinii* KU589286.



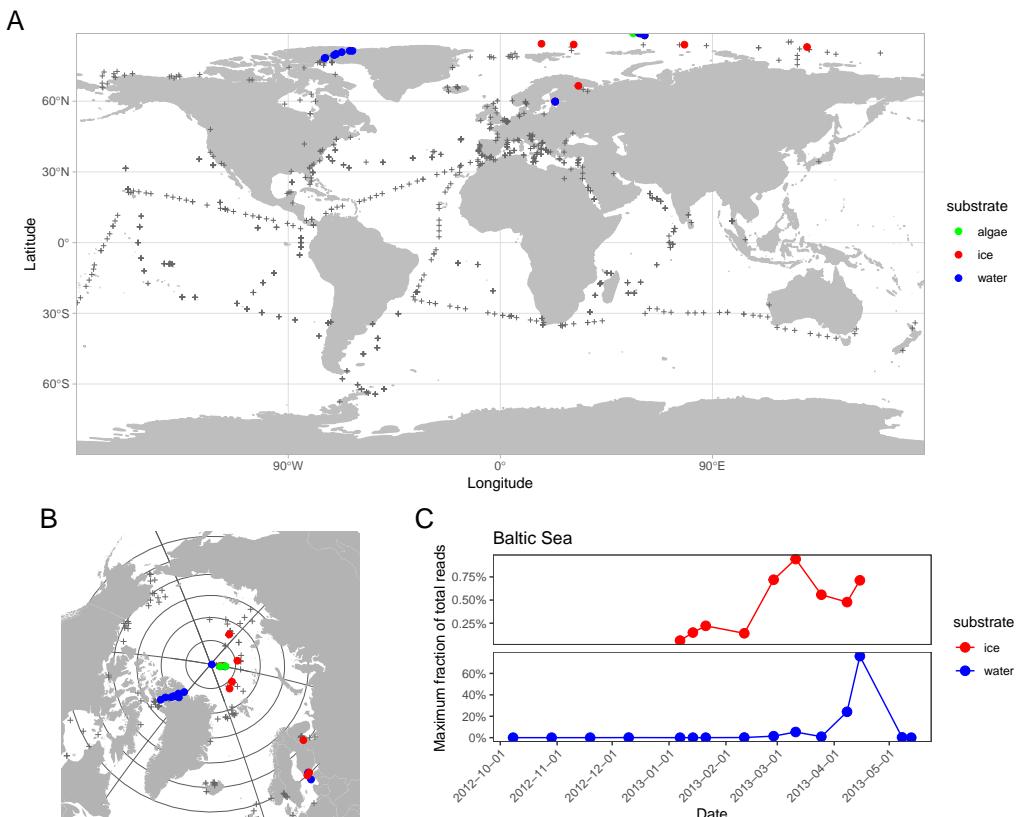
**Figure 2. Transmission electron microscopy images of RCC5270.** A) Three types of scales: small in the top, medium (short connecting bridges) in the middle and large scales in the bottom. B) Detail of a small scale with approximately 38 ribs in each quadrant. C) medium ellipsoid scale. D) Detail of the slightly curved bridge from a large scale. Scale bars have  $1 \mu\text{m}$  for A and C and  $0.5 \mu\text{m}$  for B and D.



**Figure 3.** Transmission electron microscopy images for *P. sorokinii* and *C. birgeri* modified from Orlova et al. (2016) and Hällfors & Thomsen (1979), respectively. A) *P. sorokinii* organic body scales showing three types of scales: small (arrow heads), large (arrows) and medium (white arrow, not mentioned in the description paper). B) *P. sorokinii* scales identified as small by Orlova et al. (2016), although its measurements fall within the medium scales size range, being noticeably bigger than the small scales identified in the previous image. C) *P. sorokinii* scale identified as small in the description paper; its round structure, length and width are similar to medium scales. D) *C. birgeri* image with yellow lines highlighting differences in the connecting bridge between the horn bases, the main feature used to distinguish large from medium scales in the present study. For comparison regarding size, one small scale can be seen in the upper right corner of the image. E) *C. birgeri* identified as large by Hällfors & Thomsen (1979) although its features, including the number of ribs per quadrant (54), would correspond to a medium size scale. F) *C. birgeri* true large scale, with a longer distance between horn bases and a slight curved bridge. Scale bars correspond to 1  $\mu\text{m}$  for *P. sorokinii* and no scale bar is available for *C. birgeri*.



**Figure 4.** Organic scales measurements from RCC5270 (in grey) and *P. sorokinii* independent measurements from images displayed in Orlova et al. (2016) (in blue). A) Scale length versus width; B) Scale length versus the distance between the horns. Scales visually identified as small scales are represented by squares, medium scales by triangles and large scales by circles. The size limits for *C. birgeri* described in Hällfors & Niemi (1974) and for *P. sorokinii* in Orlova et al. (2016) are displayed as yellow and blue boxes, respectively.



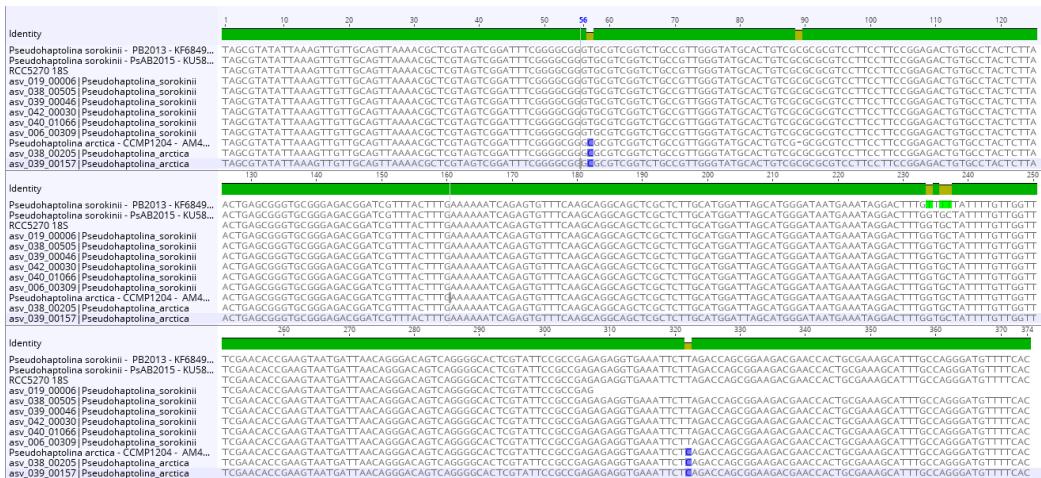
**Figure 5. RCC5270 metabarcodes.** A) Localisation of stations where 18S rRNA metabarcodes 100% identical to RCC5270 sequence have been detected in public sequence datasets (see Table 1). Color corresponds to substrate. The location of samples where these metabarcodes have not been detected are marked by grey crosses. B) Zoom on the North Pole region. C) Maximum fraction of RCC5270 metabarcodes (excluding Metazoa) as a function of date in the Gulf of Finland (Baltic Sea) in ice and water (Enberg et al., 2018).

327 **Supplementary material**

328 *Supplementary Data*

329 Supplementary data are available on GitHub at <https://github.com/vaulot/Paper->  
330 2020-Ribeiro-Pseudohaptolina

- 331 • **Supplementary Data S1:** Alignment of sequences for 18S rRNA gene  
332 (fasta file).
- 333 • **Supplementary Data S2:** Alignment of sequences for 28S (fasta file).
- 334 • **Supplementary Data S3:** Scale measurements (xlsx file).
- 335 • **Supplementary Data S4:** Number of *P. sorokinii* reads in each of the  
336 metabarcode samples analyzed (xlsx file).
- 337 • **Supplementary Data S5:** Alignment of the V4 region of the 18S rRNA for  
338 *Pseudohaptolina* reference sequences and metabarcodes (fasta file).

339 *Supplementary Figures*

**Figure S1. Partial 18S rRNA gene sequence alignment showing RCC5270 and *P. sorokinii* sequences with the *P. sorokinii* metabarcodes identified in the public datasets analyzed (Table 1).**