



HAL
open science

Transportome remodeling of a symbiotic microalga inside a planktonic host

C Juery, A Auladell, Z Füssy, F Chevalier, D P Yee, E Pelletier, E Corre, A E
Allen, D J Richter, Johan Decelle

► **To cite this version:**

C Juery, A Auladell, Z Füssy, F Chevalier, D P Yee, et al.. Transportome remodeling of a symbiotic microalga inside a planktonic host. 2024. hal-04789081

HAL Id: hal-04789081

<https://hal.science/hal-04789081v1>

Preprint submitted on 18 Nov 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 Transportome remodeling of a symbiotic 2 microalga inside a planktonic host

3 Juery C^{1*}, Auladell A², Füssy Z^{5,6}, Chevalier F¹, Yee DP¹, Pelletier E³, Corre E⁴, Allen, AE^{5,6},
4 Richter DJ², Decelle, J^{1*}

5 ¹ *Cell & Plant Physiology Laboratory, UMR 5168 CEA-CNRS-Univ. Grenoble Alpes –*
6 *UMR1417 INRAE, Grenoble, France*

7 ² *Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), Barcelona, Spain*

8 ³ *Institut de Biologie François Jacob, CEA Fontenay-aux-Roses, France*

9 ⁴ *CNRS / Sorbonne Université, Station Biologique de Roscoff, France*

10 ⁵ *Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA,*
11 *United States*

12 ⁶ *Microbial and Environmental Genomics, J. Craig Venter Institute, La Jolla, CA, United*
13 *States*

14 * correspondence: joan.decelle@univ-grenoble-alpes.fr (J.D.), caroline.juery@cea.fr (C.J.)

15

16 **Key words:** planktonic photosymbiosis – transporters – Carbon exchange – single-
17 cell transcriptomics – microalga – sugars – protists - metatranscriptomics

18

19 **COMPETING INTERESTS**

20 The authors declare no competing interests.

21

22

23

24 **ABSTRACT**

25 Metabolic exchange is one of the foundations of symbiotic associations between
26 organisms and is a driving force in evolution. In the ocean, photosymbiosis between
27 heterotrophic host and microalgae is powered by photosynthesis and relies on the
28 transfer of organic carbon to the host (e.g. sugars). Yet, the identity of transferred
29 carbohydrates as well as the molecular mechanisms that drive this exchange remain
30 largely unknown, especially in unicellular photosymbioses that are widespread in the
31 open ocean. Combining genomics, single-holobiont transcriptomics and environmental
32 metatranscriptomics, we revealed the transportome of the marine microalga
33 *Phaeocystis* in symbiosis within acantharia, with a focus on sugar transporters. At the
34 genomic level, the sugar transportome of *Phaeocystis* is comparable to non-symbiotic
35 haptophytes. By contrast, we found significant remodeling of the expression of the
36 transportome in symbiotic microalgae compared to the free-living stage. More
37 particularly, 32% of sugar transporter genes were differentially expressed. Several of
38 them, such as GLUTs, TPTs and aquaporins, with glucose, triose-phosphate sugars
39 and glycerol as potential substrates, were upregulated at the holobiont and community
40 level. We also showed that algal sugar transporter genes exhibit distinct temporal
41 expression patterns during the day. This reprogrammed transportome indicates that
42 symbiosis has a major impact on sugar fluxes within and outside the algal cell, and
43 highlights the complexity and the dynamics of metabolic exchanges between partners.
44 This study improves our understanding of the molecular players of the metabolic
45 connectivity underlying the ecological success of planktonic photosymbiosis and paves
46 the way for more studies on transporters across photosymbiotic models.

47 **INTRODUCTION**

48 Symbiosis between a heterotrophic host and a photosynthetic partner
49 (photosymbiosis) is considered to be the primary event which led to the acquisition and
50 distribution of plastids in the evolution of eukaryotes [1, 2]. Photosymbiosis remains an
51 essential life strategy and supports the functioning of today's aquatic ecosystems
52 especially in oligotrophic waters [3–6]. This partnership, considered as mutualistic in
53 the spectrum of symbioses, can provide a competitive advantage in a nutritionally
54 challenging environment where nutrients and prey are scarce (oligotrophic waters).
55 Therefore, partners need to establish a metabolic connection in order to exchange
56 metabolites and nutrients. The microalgae need to be supplied by the host with all the
57 essential macro- and micro-nutrients (e.g. iron, nitrogen) to maintain their metabolic
58 and physiological activity. In turn, the host can benefit from photosynthetic products
59 (photosynthates) exported from the microalgae [7]. In the past decade, nanoSIMS
60 (Nanoscale Secondary Ion Mass Spectrometry) studies coupled with ¹³C labeling
61 improved our knowledge on carbon transfer and allocation in different photosymbiotic
62 system [8–10]. Transferred photosynthates have been mostly investigated in benthic
63 multicellular photosymbioses such as reef-dwelling invertebrates (e.g. anemones,
64 jellyfish, giant clams) living with Symbiodiniaceae microalgae. Sugars, which are the
65 main photosynthetic products and lipids are considered to be the main photosynthates
66 exported from the symbiotic microalgae [11–13]. Glucose was shown to be a major
67 transferred metabolite in some photosymbioses [14, 15], as well as inositol, galactose,
68 and galactosylglycerol [16, 17]. Glycerol was also suggested as a putative transferred
69 metabolite since it is significantly released by free-living Symbiodiniaceae in culture
70 [18–20]. However, the exact nature of translocated carbohydrates is still uncertain

71 since experimental evidence is difficult to obtain on such photosymbiotic systems and
72 on these very rapid metabolic processes.

73 In symbiosis, both partners need to reprogram their transportome (defined as
74 “membrane proteins responsible of the translocation of any kind of solutes across the
75 lipid layer” [21]) in order to establish metabolic connectivity. Most metabolites including
76 sugars require a complete set of transporters to traverse algal and host membranes.
77 For example, in symbioses between plants and fungi, changes in expression of the
78 transporter genes are essential to connect and integrate different metabolisms [22]. In
79 marine photosymbioses, some transporters have been highlighted in genomic [23] and
80 transcriptomic studies [24]. A glucose transporter (GLUT8) and an aquaporin (GfIP)
81 that could putatively transport glycerol, were described in anemones and the jellyfish
82 *Cassiopea* [25, 26]. While most studies have focused on host transporters, less is
83 known about the ones of the symbiotic microalgae that can export energy-bearing
84 metabolites derived from photosynthesis. So far, a SWEET (Sugars Will Eventually be
85 Exported Transporter) has been described as a glucose transporter located in the cell
86 membrane of the microalga *Brevolium* (Symbiodiniaceae), symbiont of the anemone
87 *Exaiptasia diaphana* [27].

88 Relatively less studied than reef ecosystems, a wide diversity of photosymbioses are
89 also found in marine and freshwater plankton. For instance, radiolarians and
90 foraminiferans that are widespread in the sunlit layer of the ocean can host diverse
91 microalgae [28]. Among radiolarians, some species of acantharia live in symbiosis with
92 the microalga *Phaeocystis* (Haptophyta) in different oceanic regions [29]. Symbiotic
93 acantharia significantly contribute to the primary production (up to 20% in surface
94 oligotrophic oceans) and carbon fluxes to deep layers of the ocean [30, 31]. Previous
95 studies using 3D electron microscopy have shown that the microalga *Phaeocystis*

96 undergoes drastic morpho-physiological changes in symbiosis: cell and plastid volume,
97 as well as plastids number, greatly increased compared to free-living cells in culture
98 [32]. In addition, while cell division is very likely arrested in symbiosis, photosynthesis
99 and carbon fixation are enhanced, corroborated by an upregulation of many genes of
100 the Calvin-Benson cycle. Symbiotic microalgae with their expanded photosynthetic
101 apparatus therefore produce a significant amount of organic carbon but the identity of
102 these compounds and the mechanisms by which they are transferred to the host
103 remain largely unknown. Investigating the composition and expression of the algal
104 transportome can reveal how symbiotic microalgae metabolically connect to its host
105 and can provide insights on the putative exchanged metabolites.

106 Here, we conducted genomic and transcriptomic analyses on an uncultivable
107 planktonic photosymbiosis between the microalga *Phaeocystis* and acantharian hosts
108 in order to shed the light on the molecular mechanisms of their metabolic connectivity.
109 More specifically, we investigated whether the algal transportome is remodeled in
110 symbiosis. We first compared sugar transporter genes in haptophyte genomes and
111 studied their expression in free-living and symbiotic stages of *Phaeocystis* using a
112 combination of single-holobiont transcriptomics and *in situ* environmental
113 metatranscriptomics. We evaluated the transcriptional dynamics of these sugar
114 transporters in symbiosis at different periods of the day. This study reveals that the
115 transportome of the microalga is significantly remodeled in symbiosis within a host and
116 pinpoints putative key sugar transporters with different transcriptional patterns during
117 the day. This work significantly improves our understanding of the metabolic
118 connectivity between a host and microalgae and so provides fundamental knowledge
119 of the ecological success of this widespread symbiosis in the ocean.

120

121 **RESULTS AND DISCUSSION**

122 **Genomic inventory of sugar transporters in *Phaeocystis* species**

123 To understand the molecular toolbox underlying metabolic fluxes, we first unveiled the
124 transportome of the microalga *Phaeocystis* at the genomic level. We determined the
125 different categories of transporters and their proportions among six haptophytes
126 species in order to reveal a specific genomic footprint in *Phaeocystis* potentially linked
127 to its symbiotic lifestyle, compared to non-symbiotic related haptophytes (Fig. 1A). We
128 also predicted subcellular localization of transporters using a combination of different
129 *in silico* tools. We particularly focused on sugar transporters, since soluble sugars can
130 be the main exported currency to the host. Using the same method of protein
131 annotation for each species, we identified transporters based on the presence of
132 transmembrane domains and protein domain annotations using the InterPro/Pfam
133 classification. In total, we found 270 unique Pfam domains for the transporter genes in
134 haptophytes genomes. The three different *Phaeocystis* species analyzed here
135 contained an average of 965 transporter genes corresponding to 3% of all genes
136 (1,244 genes or 4% if we include those lacking a predicted transmembrane domain,
137 see Methods, Fig. 1A, Table S1, “General values1” and “General values2 (TMD)”). By
138 comparison, 2 and 3.5% of transporter genes were found in the genomes of two
139 symbiotic microalgae *Symbiodinium microadriaticum* and *Brevolium minutum*,
140 respectively (based on Joint Genome Institute’s genome portal annotations).

141 Across *Phaeocystis* species genomes, sugar transporter genes represented on
142 average 18% (175 out of 965) of all transporter genes and were classified into eight
143 different core Pfams (i.e. shared among all haptophyte species, Fig. 1A, Table S1).
144 The largest Pfam family of sugar transporters is the Triose Phosphate Transporters
145 (TPT, PF03151) with 64.7 genes on average across *Phaeocystis* species, similar to

146 *Emiliana huxleyi*, but higher than in diatom genomes (between 13 and 22 TPTs for
147 *Phaeodactylum tricornutum* [33, 34]). In plants, TPTs are transporters located in the
148 plastid envelope and export photosynthetically-derived sugars to the cytosol [35]. For
149 *P. cordata*, *in silico* subcellular localization analyses predicted only three out of 60
150 TPTs associated to the plastid membranes while the majority was predicted to be
151 located in the endomembrane system (Golgi apparatus or endoplasmic reticulum, ER)
152 (Fig. 1C Table S1). Similar to diatoms, the localization of TPTs in the four membranes
153 of the secondary red plastid of *Phaeocystis* remains ambiguous and TPTs could also
154 be localized elsewhere in the cell [36–38].

155 The second largest sugar transporter family was the “Sugar and Other transporters”
156 Pfam PF00083, containing 31 genes in *Phaeocystis* on average (41 in *Emiliana*
157 *huxleyi*, Fig. 1A). This Pfam is composed of different transporters with various
158 substrates, such as glucose, galactose, mannose, polyol, and inositol sugars [39, 40].
159 For instance, using a complementary Hidden Markov Model (HMM) search of InterPro
160 domains in *P. cordata* PF00083 proteins, we found 5 GLUT transporter (IPR002439),
161 6 Sugar transporter ERD6/Tret1-like (IPR044775), and 12 Sugar transport protein
162 STP/Polyol transporter PLT (IPR045262) domains (Table S1,
163 “GLUTcharacterization”). *In silico* subcellular localization successfully assigned a
164 prediction for 21 of the 33 transporter genes from PF00083 of *P. cordata* in the cell
165 membrane, three in vacuoles, and one in the plastid membrane (Fig. 1B). These results
166 suggest that a large part of these transporters might be involved in sugar flux at the
167 cell surface. Nucleotide sugar transporters (NSTs, PF04142) play an important role in
168 the biosynthesis of glycoproteins, glycolipids and non-cellulosic polysaccharides
169 translocating nucleotide-sugars in the Golgi [41, 42]. In *Phaeocystis* species, we found
170 34 NSTs genes on average (31 in *E. huxleyi*). In *P. cordata*, 50% of the NSTs were

171 predicted to be localized in the Golgi apparatus, in accordance with their known
172 biological function (Fig. 1C).

173 SWEET transporters (Sugars Will Eventually be Exported Transporters, PF03083) are
174 bidirectional transporters of small sugars following the concentration gradient [43].
175 SWEETs have been highlighted in terrestrial and aquatic symbioses (Fabaceae-
176 *Rhizobium*, cnidarians-Symbiodiniaceae), particularly in sugar efflux from the
177 photosynthetic to the heterotrophic partner [44–47]. Between two and four SWEET
178 genes were found across *Phaeocystis* species genomes. By comparison, many
179 duplications and diversification of SWEET genes are known in plant genomes (17 in
180 *Arabidopsis thaliana* and up to 53 in *Glycine max* [48]). Among the four SWEET
181 proteins of *P. cordata*, one was predicted in the lysosome/vacuole, one in the
182 endomembrane system, and one in the cell membrane. For the Pfam PF13347
183 (MFS/sugar transport protein) [49, 50], we found four genes in *Phaeocystis* genomes
184 In addition, we investigated the presence of putative sugar transporters from the Major
185 Facilitator Superfamily PF07690 (MFS). Through an HMM search (Table S1,
186 “PF07690_characterization”), we detected 43 putative sugar transporters for *P.*
187 *cordata* and 25 and 20 for *P. globosa* and *P. antarctica*, respectively. Among them,
188 many genes corresponded to glucose-6-phosphate transporter
189 (SLC37A1/SLC37A2, IPR044740), which are on the chloroplast membrane in plants
190 [51]. In *Phaeocystis*, most of these PF07690 transporters were predicted to be either
191 at the cell membrane or ER (Table S1, “Subcellular Localization P.cord”). Finally, we
192 investigated the presence of genes belonging to PF00230 that corresponds to a
193 specific type of aquaporin, found to be involved in different reef photosymbioses for
194 putative glycerol transport (anemones, jellyfish, and giant clams [25, 26, 52]). In

195 *Phaeocystis* genomes, seven (*P. cordata*) to nine (*P. antarctica*) homologs of these
196 aquaporin genes were found.

197 This inventory of sugar transporters in genomes unveiled different categories that
198 could be involved in the influx and efflux of sugars in the microalga *Phaeocystis*.
199 Overall, 25 sugar transporters were predicted to be localized at the cell membrane
200 (Fig. 1B), and the vast majority presented a putative localization in the endomembrane
201 system (including ER or Golgi). We also hypothesize that sugar transporters can be
202 located on vesicles derived from endomembrane system that could fuse to the cell
203 membrane [53]. We did not find any evidence of large copy number variations of sugar
204 transporter genes in *Phaeocystis* genomes compared to other haptophytes, which
205 could have been a genomic footprint to explain the predominance of this genus in
206 symbiosis. For instance, it has been demonstrated that symbiotic *Symbiodinium* clades
207 presented enriched functions related to transmembrane transport in their genomes,
208 especially for the major facilitator superfamily (PF07690) [54]. This genomic
209 characterization generates fundamental knowledge on this key marine phytoplankton
210 taxon and is an essential step for unveiling the expression dynamics of sugar
211 transporter genes in symbiosis.

212

213 **The algal transportome is significantly remodeled in symbiosis**

214 To reveal which sugar transporters might play a role in symbiosis, we assessed their
215 gene expression based on single-holobiont transcriptomic analyses. More specifically,
216 we compared the expression of transporter genes of the microalga *Phaeocystis*
217 *cordata* between free-living (four and five culture replicates in exponential and
218 stationary growth phases, respectively) and symbiotic conditions (17 holobionts
219 representing five host species collected in the Mediterranean Sea) through a

220 Differential Expression (DE) analysis (Fig. 2 and S1). Each sample was frozen at the
221 same period of the day (late evening, 7pm). About 75 million of reads were obtained
222 per holobiont sample, producing a total of 1,950 billion of reads in this study. A *de novo*
223 reference transcriptome from total RNA sequences of cultured *P. cordata* was built and
224 used to quantify gene expression.

225 Before comparing the expression of the transcriptome between the free-living and
226 symbiotic stages, we assessed whether the transcriptome of free-living *Phaeocystis*
227 cells maintained in culture varies with respect to the growth phase (i.e. exponential vs
228 stationary phase). Only 3.3% of the transporters were found to be differentially
229 expressed between exponential and stationary phase, indicating that the expression
230 of the transcriptome is not drastically modified (Table S2). We therefore considered
231 hereafter both growth phases as free-living replicates to compare with the symbiotic
232 stage.

233 From the 2133 transporter genes considered as expressed in this analysis (sum of
234 normalized read counts > 10 in all replicates, Table S3), 52% (1115 genes) were found
235 to be expressed exclusively in free-living and 12% (263 genes) exclusively expressed
236 in symbiosis (Table S3, "Exp FL Symb"). A principal component analysis (PCA) of the
237 DESeq2 dataset showing the gene expression variance revealed: i) a clear separation
238 between symbiosis and free-living samples; ii) a high variance in expression of all
239 genes across symbiotic replicates, and iii) clustering of expression of transporter genes
240 in symbiotic samples (even if a lower part of the variance -38%- is explained by the
241 first two dimensions of the PCA for transporter genes compared to all genes -74%-,
242 Fig. 2A and 2B). We verified this clustering pattern by using different subsets of
243 expressed genes with similar numbers of genes as controls (Fig. S1). These results

244 show the existence of two distinct transportomes of the microalga *Phaeocystis*
245 expressed in the symbiotic and free-living stages.

246 Compared to the free-living stage, 42% of the transportome was significantly
247 remodeled in symbiosis with 888 differentially expressed genes (Fig 2C). Among
248 those, 26% (556 genes) were downregulated and 16% (332 genes) were upregulated
249 in symbiosis (Table S3, “UP-DOWN global”). These numbers are higher than the ones
250 found in the symbiotic *Brevolium*, and with an opposite trend: 213 up-regulated and
251 167 down-regulated [24]. We explain this remodeling of *Phaeocystis* transportome in
252 symbiosis by a significant global decrease of the transporter gene expression
253 (normalized read counts, Wilcoxon rank sum test, p-value <0.01, Fig. 2C) and high
254 positive fold changes for some transporter genes (Fig 2D, aquaporin PF00230 with
255 log₂FC = 13.58, Table S3). Note that 58% of the algal transportome remained
256 expressed in symbiosis but without differential expression. Therefore, these results
257 suggest that many algal transporters could be less required in symbiosis likely due to
258 the transition from the ocean to the host microhabitat, but some transporters could be
259 specifically induced in symbiosis with high transcriptional activity in response to
260 metabolic changes within a host and potentially enable the metabolic connectivity
261 between the two partners.

262

263 **Expression of the algal sugar transporters in symbiosis**

264 We next focused on the expression of sugar transporter genes of the microalga in
265 symbiosis. From our dataset, 223 genes were found to be expressed (out of the 330
266 sugar transporter genes in the reference transcriptome; TableS3 “UP-DOWN global“
267 and “*P.cordata* SugarTR Anno”). At the Pfam level, no significant changes of the global
268 gene expression of a family were observed between free-living and symbiotic

269 microalgae (Fig. 3A). Yet, at the gene level, 32% of sugar transporters were remodeled
270 in symbiosis with 9 % (19 genes) upregulated and 23% (52 genes) downregulated (Fig.
271 2D, Table S3, “UP-DOWN global”). Note that 68% (152 genes out of the 223) of the
272 sugar transporter genes were still expressed in symbiosis (named “neutral” in Table
273 1). Among the 19 up-regulated genes in symbiosis, we found ten TPTs (Triose
274 Phosphate Transporters, PF03151, average log₂FC = 6.4), two Aquaporins (PF00230,
275 average log₂FC = 14.4), two monosaccharide transporters (PF00083, average log₂FC
276 = 4.3), one MFS/sugar transport protein (PF13347, average log₂FC = 5.7), two
277 Nucleotide sugar transporters (PF04142, average log₂FC = 5.4) and two genes from
278 PF07690 (annotated as Glycerol-3-P transporter, average log₂FC = 4.1) (Fig. 3B,
279 Table 1). These transporters therefore participate to the significant remodeling and
280 specialization of the algal sugar transportome in symbiosis and potentially play a key
281 role in the flux and exchange of sugars.

282 Concerning the TPTs, only 10 out of 85 genes (12%) were upregulated and 66% were
283 still expressed in symbiosis. It is possible that the observed upregulation of some TPTs
284 can be linked to the multiplication of plastids in symbiosis (from two in free-living to up
285 to 60 plastids in symbiosis [32]) and would ensure enhanced sugar export into the
286 cytosol. The most upregulated sugar transporter genes correspond to two aquaporins
287 (PF00230, log₂FC of 15.1 and 13.6) (Fig. 3B, Table 1, Table S3). This type of
288 aquaporin, also known as GlpF (Glycerol Facilitator), was found to be involved in two
289 benthic photosymbioses and suggested to be involved in glycerol transport [25, 26].
290 The four upregulated transporter genes with glycerol as putative substrate (two genes
291 of PF00230 and two genes of PF07690) raise the hypothesis that this metabolite can
292 be important for the carbon metabolism of the holobiont and possibly transported to
293 the host. Two other upregulated genes corresponded to putative transporters of

294 monosaccharides (PF00083), with assignment to GLUT proteins (Fig. S2 and Table
295 S1, “GLUT Characterization”). One of these two GLUT proteins was predicted to be
296 localized at the cell membrane (Table S3, “SubcellLoc SugarTRUP”). Four SWEET
297 transporter genes (PF03083) were expressed in symbiosis (log₂FC ranging between
298 -1.5 to 0.90), but not upregulated. The upregulated MFS/sugar transport protein
299 (PF13347, log₂FC = 5.7) could also play a role in sugar flux as shown in plants [50].
300 This transcriptomic analysis from freshly collected holobionts allowed us to reveal the
301 sugar transportome expressed in the symbiotic microalga and identify candidate genes
302 for future functional characterization. To have an alternative line of evidence, we
303 investigated the expression of this algal transportome *in situ*, exploiting
304 metatranscriptomic data collected in the Mediterranean Sea.

305

306 ***In situ* sugar transportome expression of the microalga *Phaeocystis* in the** 307 **Mediterranean Sea**

308 Using the *Tara* metatranscriptomic dataset from the Mediterranean Sea, we evaluated
309 the expression of sugar transporter genes of the microalga *Phaeocystis* in two size
310 fractions (small: 0.8–5 µm; and large: 180–2000 µm) collected in surface waters of six
311 stations from the *Tara* Oceans expedition ([55], Fig. 4A). The small size fraction mainly
312 corresponds to the free-living stage of *Phaeocystis cordata* (4 µm in size [56]) while its
313 symbiotic stage within acantharians is mainly detected in the large size fraction (note
314 that the Mediterranean species, *Phaeocystis cordata*, does not form colonies [57]).
315 From this metatranscriptomic dataset, we identified 270 sugar transporters with a
316 difference between the large (123 genes) and small (266 genes) size fraction. This can
317 be partially explained by the lower abundance of *Phaeocystis* transcripts in the 180-

318 2000 μm fraction that might have been diluted and so less sequenced due to the high
319 abundance of transcripts from large multicellular organisms (zooplankton) (Fig. 4B).
320 In order to normalize and compare gene expression between the two size fractions,
321 we calculated the ratio between the expression (TPM) of the 270 sugar transporter
322 genes and a selected set of 149 housekeeping genes (e.g. Ribosomal proteins,
323 Tubulin, ATP synthase...) whose expression levels were expected to correspond to
324 basic cellular activity (see Methods and Table S4). Based on this normalization
325 method, we found 26 sugar transporter genes with a higher mean expression value in
326 the large size fraction compared to the small one: twelve TPTs, six NSTs, three
327 aquaporins, two Glycerol-Phosphate transporters, one GLUT, one SWEET and one
328 member for the MFS/sugar transporter protein PF13347 (Fig. 4B, Table S4). Fourteen
329 out of the nineteen sugar transporter genes found as upregulated in symbiosis (Fig.3B,
330 single-holobiont transcriptomic analysis) were also found as expressed in both size
331 fractions of the metatranscriptomic dataset. Of these, six of them presented a higher
332 TPM value in the large size fraction: two aquaporin PF00230 genes, one of the two
333 GLUT and three TPTs (Fig. 4C, Table S4). Note that the two aquaporin genes identified
334 here correspond to the genes that presented the highest positive fold change values
335 in symbiosis in the differential expression analysis from isolated holobionts (Fig. 3B).
336 This *in situ* metatranscriptomic analysis provides an ecological significance to our
337 experimental results, and further confirm some sugar transporter candidates as key
338 players in symbiosis, such as TPTs, GLUT and aquaporin.

339

340 **Dynamic expression of the algal sugar transportome in symbiosis during the**
341 **day**

342 It is well established that photosynthesis and the central carbon metabolism depend
343 on the circadian rhythm and light conditions [58–60]. Therefore, in order to further
344 understand the metabolic connectivity, we investigated the transcriptional dynamics of
345 the sugar transportome of symbiotic *Phaeocystis* by harvesting acantharian hosts (n =
346 4) at three different times of day and light exposure periods: 1) morning (9 am, after 1h
347 light exposure), 2) evening (7 pm, after 10h light exposure) and 3) “dark-evening” (7pm,
348 after an incubation in darkness for 24 hours). The “dark-evening” condition
349 corresponds to a situation where holobionts, and thus microalgae experienced a non-
350 photosynthetic day (absence of light). In total, we found 209 sugar transporter genes
351 expressed in these three conditions, representing 96% (214/223) of the sugar
352 transportome characterized above (Table S5, Table 1). Overall, 28% (60 genes) of
353 sugar transporters were found to be expressed in the morning, 43% (91 genes)
354 expressed in the evening and 29% (63 genes) in the “dark-evening” (Table S5). Among
355 them, some were exclusively expressed in the morning (18), evening (43) or dark-
356 evening (16) (Fig. 5A, Table S5). These results demonstrate that many sugar
357 transporter genes of the symbiotic microalga tend to be induced during the day. From
358 the comparison of holobionts collected in the evening and submitted or not to darkness
359 (“dark-evening”), we found 53% (113/214) of genes downregulated when incubated in
360 the dark (FC < -2, Table S5). 34% (72/214) were still expressed in the holobionts
361 exposed to darkness and thus, their expression does not seem to be linked to the
362 presence of light. These results show that the majority of sugar transporter expression
363 of the symbiotic microalga is modulated by light conditions.

364 At the Pfam level, we found specific expression patterns modulated by either light or
365 period of the day (Fig. 5B, Table S5). For instance, the expression of TPTs and
366 Glycerol 3-Phosphate transporter (PF07690) tended to be modulated only by light
367 since they were significantly more expressed in evening vs “dark-evening” condition
368 and did not show significantly higher expression between morning and evening
369 (Wilcoxon rank sum test, p -value <0.05 , Fig. 5B, Table S5). On the contrary,
370 transcription of sugar hexose transporters (PF00083) seemed to be more regulated by
371 the period of the day as shown by a significant higher expression in the evening
372 compared to morning but not differentially expressed between evening and “dark-
373 evening” conditions (Wilcoxon p -value <0.05 , Fig. 5B, Table S5). Nucleotide sugar
374 transporters (NSTs) seemed to be regulated by both parameters (light and period of
375 the day) as they were found to be significantly more expressed in the evening vs
376 morning, and “dark-evening” vs evening (Wilcoxon p -value <0.01 , Fig. 5B, Table S5).
377 MFS/sugar transport protein (PF13347) genes tended to present a higher expression
378 in the dark (Fig. 5B, Table S5). SWEET genes did not present significant differences
379 of expression levels between the three conditions, yet two pairs of genes seemed to
380 present opposite patterns (more expressed in the morning or in the dark). Generally,
381 aquaporin genes (PF00230) exhibited a lower expression in the darkness and two of
382 them were only expressed in the evening, after the normal daylight exposure. These
383 results show specific transcriptional patterns of sugar transporters according to each
384 Pfam families responding to light (PF07690, PF03151, PF13347), to the period of the
385 day (PF00083) or both parameters (PF04142).

386 We also paid attention to the dynamics of the sugar transporter genes found to be
387 upregulated in symbiosis from our holobiont transcriptomes. The ten upregulated TPTs
388 in symbiosis exhibited several transcriptional patterns: two genes exclusively

389 expressed in the morning and two genes exclusively in the evening; in addition, two
390 gene had a higher expression in the evening compare to morning and six a higher
391 expression in light (evening) compare to dark-evening (Fig. 5C, Table S5). This
392 suggests that different TPT genes might have specific roles at different periods of the
393 day and this could depend on their subcellular localization. Most GLUT and aquaporin
394 genes upregulated in symbiosis showed a higher expression in morning vs evening, or
395 “dark-evening” vs evening, raising two hypotheses: 1) the transcription is activated in
396 the morning to produce transporters during the day or 2) transcription mainly takes
397 place in the dark for sugar excretion at night. Further studies should increase the
398 temporal resolution during a day-night cycle to fully reveal the dynamics of the
399 transportome expression of the symbiotic microalga.

400 **CONCLUSION AND PERSPECTIVES**

401 This study improves our understanding of the molecular players that are potentially
402 involved in the carbon metabolism and metabolic connectivity between the symbiotic
403 microalga *Phaeocystis* and its acantharian host. We found that *Phaeocystis* species
404 share a conserved sugar transportome among haptophytes at the Pfam level with few
405 differences in gene copy number. Therefore, this genomic analysis did not reveal a
406 specific sugar transportome linked to the symbiotic life stage of the microalga
407 *Phaeocystis*, compared to non-symbiotic haptophytes. This can be explained by the
408 fact that *Phaeocystis* symbionts are not vertically transmitted across host generations,
409 do not depend on symbiosis for survival, and genome evolution would rather occur in
410 the extensive free-living population [5]. Our study shows that the capacity of the
411 microalga *Phaeocystis* to be in symbiosis may be rather due to the large plasticity of
412 the transportome expression with 42% of transporter genes being differentially
413 expressed. This suggests a drastic change in the flux and homeostasis of metabolites

414 in the symbiotic microalgae. More specifically, downregulation of most transporter
415 genes along with high expression of a few ones suggests i) lower trafficking of
416 metabolites linked to the intracellular life stage (perhaps due to the arrested cell
417 division) ii) specialization towards some metabolite fluxes putatively beneficial for the
418 host. The transcriptional plasticity of the algal sugar transportome not only takes place
419 during the free-living-to-symbiosis transition but also throughout the day. This reveals
420 the complex dynamics of the carbon homeostasis and fluxes in the holobiont system.

421 Among the 19 sugar transporters of the microalga *Phaeocystis* upregulated in
422 symbiosis, we found two GLUT and two aquaporin genes, which were also found more
423 expressed in the large size fraction of environmental metatranscriptomics. This study
424 provides further evidence that GLUT and aquaporin transporters, also found as
425 upregulated in other photosymbiotic hosts [25, 26], play a key role in symbiosis.
426 Similarly, the higher expression of a SWEET gene in this large size fraction suggests
427 that this transporter may also be involved in metabolic connectivity in this planktonic
428 symbiosis, as shown for anemone/dinoflagellate symbiosis [44].

429 The diversity of transporters and their expression patterns raise the hypothesis that
430 several algal carbohydrates (glucose, glycerol) might be transferred to the host at
431 different temporal windows. Future functional characterization (e.g. expression in
432 heterologous systems) of the candidate sugar transporters revealed here will be
433 essential to fully understand the role of these transporters in symbiosis. Overall, this
434 study expands the list of holobionts using similar transporter genes and raises the
435 hypothesis of a convergence for carbon exchange mechanisms in photosymbiosis.

436 **MATERIAL AND METHODS**

437 **Dataset of genomic sequences of haptophytes transporters**

438 Genomic identification of transporter genes was obtained from a re-annotation of the
439 186,115 protein sequences of six haptophyte species (*Diacronema lutheri*, *Emiliania*
440 *huxleyi*, *Chrysochromulina tobinii*, *Phaeocystis antarctica*, *Phaeocystis globosa*,
441 *Phaeocystis cordata*) from Phycocosm [61]. Sequences were re-annotated with
442 multiple tools in order to have a complete description of each transporter useful for
443 downstream analysis: Interproscan 5.60 with best scores for E-values < 0.000001 [62],
444 TCDB Transporter Classification Database [63], blastp of the protein sequences with
445 diamond (2.1.7, options: -e 0.00001 --ultra-sensitive --max-target-seqs 1) to the
446 Uniprot release 2022 02, Hmmscan with Pfam-A.hmm 2021-11-15 [64] , EggNog
447 mapper [65]. To identify transporters, we first search in the merged files of annotations
448 for the terms:
449 "carrier|transport|channel|permease|symporter|exchanger|antiporter|periplasmic|facili
450 tator". We search sugar transporters using terms such as:
451 "disacchahride|carbohydrate|sugar|ose|saccharide|glucose|polysaccharide".

452 We used Phobius [66] and tmhmm 2.0 [67] to predict the number of transmembrane
453 domains. We selected proteins that presented at least two transmembrane domains
454 and less than three differences in terms of transmembrane domain number between
455 Phobius and tmhmm. For PF00083 and PF07690 families, we used protein models
456 (from InterPro database, <https://www.ebi.ac.uk/interpro/>) of the subfamilies domains
457 described in Table S1, in order to build hmm profiles and use hmmsearch (best value,
458 evaluate E-23) to precise the identity of these transporters. Subcellular localization of *P.*
459 *cordata* transporters was evaluated through five different tools: Deeploc 2.0 [68],

460 TargetP 2.0 [69], Hectar 1.3 [70], WoLF PSORT [71], MuLocDeep 1 [72]. We used two
461 thresholds 1) a Deeploc score >0.5 (as used in [73]) and 2) the consistency of
462 prediction should be least the same for at least two tools, to select the most accurate
463 putative predictions of transporters localization.

464 **Single-holobiont transcriptomics: sampling and analysis**

465 For the free-living stage, a total of nine replicates of *Phaeocystis cordata* cells (strain
466 RCC1383 from the Roscoff Culture Collection) maintained in K2 medium at 50-60 μmol
467 PAR $\text{m}^{-2}\text{s}^{-1}$ and 20°C were harvested at 7pm at both late and stationary growth stages.
468 Symbiotic acantharians with intra-cellular *Phaeocystis cordata* (holobionts) were
469 collected with a 150 μm plankton net in Mediterranean Sea in Villefranche-sur-Mer,
470 France. Individual holobionts were manually isolated with a micropipette under a
471 binocular microscope and rapidly transferred into filtered seawater (0.2 μm) and
472 maintained in an incubator (50-75 μmol PAR $\text{m}^{-2}\text{s}^{-1}$, 20°C, 12h/12h). Free-living and
473 symbiotic samples were frozen in the same conditions, in liquid nitrogen in a 0.2 μl
474 PCR tube containing 4.4 μl of Smart-Seq2 buffer (Triton X-100 0.4 %/RNase inhibitor
475 Ratio 19/1, dNTPs 10 mM, oligo dT 5 μM , [74]). Each sample was sequenced at 75
476 million reads, 2 \times 150 paired-end with an Illumina NextSeq 500 instrument. A total of 1.9
477 billion reads were produced for this study.

478 Reads were first trimmed using trimmomatic (version 0.39, option PE -phred33;
479 ILLUMINACLIP: contams_forward_rev.fa:2:30:10 LEADING:3 TRAILING:3
480 SLIDINGWINDOW:4:15 MINLEN:36 [75]) and bacterial, virus, human, fungi
481 sequences contaminants were removed using kraken2 (2.1.2) and the
482 k2_standard_202310 database [76]. In order to maximize the mapping rates of the
483 reads, we built a new reference transcriptome of *Phaeocystis cordata* from the reads
484 obtained with the sequencing of our culture (strain RCC1383 from the Roscoff Culture

485 Collection) plus the reads from the PRJNA603434 BioProject deposited at NCBI
486 GenBank [32] from the same *Phaeocystis* strain. Briefly, reads were assembled using
487 rnaSPAdes v3.15.5 [77] and peptides were predicted using TransDecoder [78]. The
488 peptides were annotated with the same method as for the genomic proteome.
489 Conserved ortholog scores were calculated with BUSCO v5.4.4 [79] and alignment
490 rates with Bowtie2 [80]. We reached a 68.87% re-mapping rate of the reads (31.13%
491 with the previous reference, Figure S3); the two transcriptome references (this study
492 and [32]) presented the same completeness (BUSCO score, Fig. S3 and [32]).

493 To verify if the decontamination of the reference transcriptome step using kraken2 was
494 sufficient, we applied two blastp searches of the predicted peptides (as queries): 1)
495 against *P. cordata* protein sequences from the Joint Genome Institute's genome portal;
496 and 2) against the NCBI nr database. For the 330 transcripts annotated as sugar
497 transporter in the reference transcriptome, 96% of them were found in the protein
498 sequences of *P. cordata* genome. The contigs presenting <50% identity (4 contigs)
499 have a NCBI blast with *Oryza sativa*, *Arabidopsis thaliana* and 2 Bacteria but with a
500 percentage of identity very close to the one found with the blastp against *P. cordata*
501 genome-derived protein models. For the 14 proteins not found in *P. cordata* genome,
502 only 4 have a NCBI match with a bacterial assignment but again with a <50% of
503 identity. Thus, in total, six sugar transporters genes putatively presented a bacterial
504 homolog but with ~35% identity on average (Table S2, "merged_blastp").

505 For the Differential Expression (DE) analysis, read counts were obtained using Kallisto
506 (0.48.0) [81] to map the reads from holobionts and free-living RNA sequences on the
507 reference transcriptome coding sequences. The differential gene expression analysis
508 between free-living vs symbiotic stages was conducted using the DESeq2 R package

509 (1.36.0, [82]). We used the threshold of normalized read counts >10 among all
510 replicates to qualify a gene as expressed in a given condition [32].

511 **Tara Oceans metatranscriptomic data analysis**

512 Reads of the Mediterranean stations 011, 009, 022, 023, 025 and 030 of the *Tara*
513 Oceans expedition (2014) were obtained from the published dataset
514 (PRJEB402/ERP006152, [55]). To compare the expression of sugar transporter genes
515 between two size fractions, we transformed their TPM values into ratios between the
516 expression of genes of interest and housekeeping genes that we identified in a similar
517 way to the approach used by qPCR (Table S4 “HousekeepingGenes”). Briefly, the
518 community expression patterns are compositional data, not absolute counts, and this
519 type of data is constrained to an arbitrary fixed total defined by the sequencing depth,
520 creating potentially spurious correlations through changes in abundance of other
521 organisms in the system (see [83] for a review on the topic). A solution to this problem
522 is to analyze ratios of gene expression instead of the proportions of the total. In this
523 approach, choosing an appropriate denominator (housekeeping genes) to calculate
524 ratios is critical [84]. We established a robust denominator via 4 criteria: (1) analyzing
525 only samples in which at least 20% of the *Phaeocystis* transcriptome was expressed,
526 and retain only genes expressed in all samples showing at least 20% *Phaeocystis*
527 expression in every analyzed sample (this procedure eliminated 7 of the top 20 genes
528 with the most reads mapped, likely because their high expression was the result of
529 non-specific mapping in samples in which less than 20% of the *Phaeocystis*
530 transcriptome was expressed), (2) selecting genes whose expression across all
531 analyzed samples has a coefficient of variation below 200 (mean CV all genes= 374.8)
532 and a fold change from the mean below 2, (3) checking that the genes present a
533 functional annotation corresponding to typical housekeeping genes, and (4) checking

534 that the genes correlate enough between them and through a k-means
535 clustering, selecting the cluster with the highest amount of genes presenting $r > 0.5$
536 (adapting [84]). We then used the sugar transporters' TPM as a numerator and the
537 geometric mean of the housekeeping selected genes as a denominator to calculate
538 our statistics.

539 **Temporal transcriptional dynamics of sugar transporters of the microalga**

540 ***Phaeocystis cordata***

541 To evaluate the expression of the sugar transportome during the day, we collected
542 more symbiotic acantharia in surface waters of the Mediterranean Sea (Villfranche-
543 sur-Mer, France). For “Morning” samples, we sampled and isolated acantharia,
544 maintained them in an incubator overnight (50-75 $\mu\text{mol PAR m}^{-2}\text{s}^{-1}$, 20°C, 12h/12h),
545 and harvested them the day after at 9 am in the morning (one hour of light exposure).
546 For “evening” samples, symbiotic acantharia were collected and isolated in filtered
547 seawater, and frozen the same day around 7pm in the evening after 10 hours of light
548 in the incubator. For “dark-evening” samples, holobionts were collected and
549 maintained in the incubator with light until 8pm, and were transferred in a black box
550 until 7pm of the following day. In order to compare gene expression across time/light
551 conditions (morning, evening and dark-evening), we built a matrix of read counts for
552 the three conditions using kallisto on the reference transcriptome of *P. cordata*, as
553 explained above, and normalizing these counts using DESeq2 R package without
554 “reference level” for the dds object creation.

555 **ACKNOWLEDGMENTS**

556 Research was supported by CNRS and ATIP-Avenir program funding, and by the
557 European Union (BIOcean5D - GA#101059915 and ERC – SymbiOCEAN -

558 101088661). We thank the EMBRC program and the Institut de la mer de Villefranche-
559 sur-Mer (IMEV, France) for the sampling. This project has received funding from the
560 European Union's Horizon 2020
561 research and innovation programme under grant agreement No 824110 for the
562 sequencing (EASI Genomics). We thank Annelien Verfaillie and Alvaro Cortes from
563 the Genomics Core platform in Leuven. A. Auladell and D. J. Richter received funding
564 from the European Research Council (ERC) under the European Union's Horizon 2020
565 research and innovation program (grant agreement 949745 — GROWCEAN — ERC-
566 2020-STG) and from the Departament de Recerca i Universitats de la Generalitat de
567 Catalunya (exp. 2021 SGR 00751). Z. Füßy and A. Allen were funded by National
568 Oceanic and Atmospheric Administration grant NA19NOS4780181 (to AEA), the
569 National Science Foundation (NSF-OCE-1756884 to AEA), and the Simons
570 Collaboration on Principles of Microbial Ecosystems (PriME) (Grant ID: 970820 to
571 AEA). The work (proposal: 10.46936/10.25585/60001426) conducted by the U.S.
572 Department of Energy Joint Genome Institute (<https://ror.org/04xm1d337>), a DOE
573 Office of Science User Facility, is supported by the Office of Science of the U.S.
574 Department of Energy operated under Contract No. DE-AC02-05CH11231. We are
575 grateful to the Roscoff Bioinformatics platform ABiMS (<http://abims.sb-roscoff.fr>), part
576 of the Institut Français de Bioinformatique (ANR-11-INBS-0013) and BioGenouest
577 network, for providing help for computing HECTAR subcellular localization prediction.
578 We also thank Margaret Mars Brisbin for her help on the bioinformatic analyses.

579

580 **AUTHOR CONTRIBUTIONS**

581

582 CJ and JD conceived, planned the research and wrote the manuscript; CJ, JD and FC
583 performed sampling and experiments sampling; CJ performed data analysis, figures
584 generation and drafted the first manuscript; AA, EP and DR performed data analysis
585 from metatranscriptomics of *Tara* data. All authors participated in discussions
586 interpreting results and provided comments and approved the final version.

587

588 **DATA AVAILABILITY**

589 All raw sequencing data generated for this manuscript are available from the NCBI SRA
590 under accession XXXXXXXXXX.

591 **REFERENCES**

- 592 1. Archibald JM. Genomic perspectives on the birth and spread of plastids. *Proc Natl Acad*
593 *Sci* 2015; **112**: 10147–10153.
- 594 2. Keeling PJ. The endosymbiotic origin, diversification and fate of plastids. *Philos Trans R*
595 *Soc B Biol Sci* 2010; **365**: 729–748.
- 596 3. Burki F, Sandin MM, Jamy M. Diversity and ecology of protists revealed by
597 metabarcoding. *Curr Biol* 2021; **31**: R1267–R1280.
- 598 4. Decelle J, Stryhanyuk H, Gallet B, Veronesi G, Schmidt M, Balzano S, et al. Algal
599 Remodeling in a Ubiquitous Planktonic Photosymbiosis. *Curr Biol CB* 2019; **29**: 968-
600 978.e4.
- 601 5. Decelle J. New perspectives on the functioning and evolution of photosymbiosis in
602 plankton. *Commun Integr Biol* 2013; **6**: e24560.
- 603 6. de Vargas C, Audic S, Henry N, Decelle J, Mahé F, Logares R, et al. Eukaryotic
604 plankton diversity in the sunlit ocean. *Science* 2015; **348**: 1261605.
- 605 7. Davy SK, Allemand D, Weis VM. Cell Biology of Cnidarian-Dinoflagellate Symbiosis.
606 *Microbiol Mol Biol Rev* 2012; **76**: 229–261.
- 607 8. LeKieffre C, Jauffrais T, Geslin E, Jesus B, Bernhard JM, Giovani M-E, et al. Inorganic
608 carbon and nitrogen assimilation in cellular compartments of a benthic kleptoplastic
609 foraminifer. *Sci Rep* 2018; **8**: 10140.
- 610 9. Kopp C, Wisztorski M, Revel J, Mehiri M, Dani V, Capron L, et al. MALDI-MS and
611 NanoSIMS imaging techniques to study cnidarian–dinoflagellate symbioses. *Zoology*
612 2015; **118**: 125–131.

- 613 10. Decelle J, Veronesi G, LeKieffre C, Gallet B, Chevalier F, Stryhanyuk H, et al.
614 Subcellular architecture and metabolic connection in the planktonic photosymbiosis
615 between Collodaria (radiolarians) and their microalgae. *Environ Microbiol* 2021; **23**:
616 6569–6586.
- 617 11. Catacora-Grundy A, Chevalier F, Yee D, LeKieffre C, Schieber NL, Schwab Y, et al.
618 Sweet and fatty symbionts: photosynthetic productivity and carbon storage boosted in
619 microalgae within a host. *BioRxiv* 2023
- 620 12. Hambleton EA. How corals get their nutrients. *eLife* 2023; **12**: e90916.
- 621 13. Papina M, Meziane T, van Woesik R. Symbiotic zooxanthellae provide the host-coral
622 *Montipora digitata* with polyunsaturated fatty acids. *Comp Biochem Physiol B Biochem*
623 *Mol Biol* 2003; **135**: 533–537.
- 624 14. Markell DA, Trench RK. Macromolecules Exuded by Symbiotic Dinoflagellates in
625 Culture: Amino Acid and Sugar Composition1. *J Phycol* 1993; **29**: 64–68.
- 626 15. Burriesci MS, Raab TK, Pringle JR. Evidence that glucose is the major transferred
627 metabolite in dinoflagellate–cnidarian symbiosis. *J Exp Biol* 2012; **215**: 3467–3477.
- 628 16. Ishii Y, Ishii H, Kuroha T, Yokoyama R, Deguchi R, Nishitani K, et al. Environmental pH
629 signals the release of monosaccharides from cell wall in coral symbiotic alga. *eLife*
630 2023; **12**: e80628.
- 631 17. Gordon BR, Leggat W. Symbiodinium—Invertebrate Symbioses and the Role of
632 Metabolomics. *Mar Drugs* 2010; **8**: 2546–2568.
- 633 18. Muscatine L. Glycerol Excretion by Symbiotic Algae from Corals and *Tridacna* and Its
634 Control by the Host. *Science* 1967; **156**: 516–519.
- 635 19. Hillyer KE, Dias DA, Lutz A, Wilkinson SP, Roessner U, Davy SK. Metabolite profiling of
636 symbiont and host during thermal stress and bleaching in the coral *Acropora aspera*.
637 *Coral Reefs* 2017; **36**: 105–118.
- 638 20. Suescún-Bolívar LP, Traverse GMI, Thomé PE. Glycerol outflow in Symbiodinium under
639 osmotic and nitrogen stress. *Mar Biol* 2016; **163**: 128.

- 640 21. Ruffinatti FA, Scarpellina G, Chinigo G, Visentin L, Munaron L. The Emerging Concept
641 of Transportome: State of the Art. **Physiology** 2023; **38**: 285-302.
- 642 22. Banasiak J, Jamruszka T, Murray JD, Jasiński M. A roadmap of plant membrane
643 transporters in arbuscular mycorrhizal and legume–rhizobium symbioses. *Plant Physiol*
644 2021; **187**: 2071–2091.
- 645 23. Sproles AE, Kirk NL, Kitchen SA, Oakley CA, Grossman AR, Weis VM, et al.
646 Phylogenetic characterization of transporter proteins in the cnidarian-dinoflagellate
647 symbiosis. *Mol Phylogenet Evol* 2018; **120**: 307–320.
- 648 24. Maor-Landaw K, van Oppen MJH, McFadden GI. Symbiotic lifestyle triggers drastic
649 changes in the gene expression of the algal endosymbiont *Breviolum minutum*
650 (*Symbiodiniaceae*). *Ecol Evol* 2020; **10**: 451–466.
- 651 25. Mashini AG, Oakley CA, Grossman AR, Weis VM, Davy SK. Immunolocalization of
652 Metabolite Transporter Proteins in a Model Cnidarian-Dinoflagellate Symbiosis. *Appl*
653 *Environ Microbiol* 2022; **88**: e00412-22.
- 654 26. Carabantes N, Grosso-Becerra MV, Thomé PE. Expression of glucose (GLUT) and
655 glycerol (GLP) transporters in symbiotic and bleached *Cassiopea xamachana* (Bigelow,
656 1892) jellyfish. *Mar Biol* 2024; **171**: 54.
- 657 27. Maor-Landaw K, Eisenhut M, Tortorelli G, van de Meene A, Kurz S, Segal G, et al. A
658 candidate transporter allowing symbiotic dinoflagellates to feed their coral hosts. *ISME*
659 *Commun* 2023; **3**: 1–7.
- 660 28. Decelle J, Colin S, Foster RA. Photosymbiosis in Marine Planktonic Protists. In:
661 Ohtsuka S, Suzaki T, Horiguchi T, Suzuki N, Not F (eds). *Marine Protists: Diversity and*
662 *Dynamics*. 2015. Springer Japan, Tokyo, pp 465–500.
- 663 29. Decelle J, Probert I, Bittner L, Desdevises Y, Colin S, de Vargas C, et al. An original
664 mode of symbiosis in open ocean plankton. *Proc Natl Acad Sci* 2012; **109**: 18000–
665 18005.
- 666 30. Poff KE, Leu AO, Eppley JM, Karl DM, DeLong EF. Microbial dynamics of elevated
667 carbon flux in the open ocean’s abyss. *Proc Natl Acad Sci* 2021; **118**: e2018269118.

- 668 31. Michaels AF. Vertical distribution and abundance of Acantharia and their symbionts.
669 *Mar Biol* 1988; **97**: 559–569.
- 670 32. Uwizeye C, Mars Brisbin M, Gallet B, Chevalier F, LeKieffre C, Schieber NL, et al.
671 Cytoklepty in the plankton: A host strategy to optimize the bioenergetic machinery of
672 endosymbiotic algae. *Proc Natl Acad Sci* 2021; **118**: e2025252118.
- 673 33. Liu S, Storti M, Finazzi G, Bowler C, Dorrell RG. A metabolic, phylogenomic and
674 environmental atlas of diatom plastid transporters from the model species
675 *Phaeodactylum*. *Front Plant Sci* 2022; **13**.
- 676 34. Moog D, Nozawa A, Tozawa Y, Kamikawa R. Substrate specificity of plastid phosphate
677 transporters in a non-photosynthetic diatom and its implication in evolution of red alga-
678 derived complex plastids. *Sci Rep* 2020; **10**: 1167.
- 679 35. Fischer K. The Import and Export Business in Plastids: Transport Processes across the
680 Inner Envelope Membrane1. *Plant Physiol* 2011; **155**: 1511–1519.
- 681 36. Moog D, Rensing SA, Archibald JM, Maier UG, Ullrich KK. Localization and Evolution of
682 Putative Triose Phosphate Translocators in the Diatom *Phaeodactylum tricornutum*.
683 *Genome Biol Evol* 2015; **7**: 2955–2969.
- 684 37. Facchinelli F, Weber AP. The Metabolite Transporters of the Plastid Envelope: An
685 Update. *Front Plant Sci* 2011; **2**.
- 686 38. Fischer K, Weber A. Transport of carbon in non-green plastids. *Trends Plant Sci* 2002;
687 **7**: 345–351.
- 688 39. Misra VA, Wafula EK, Wang Y, dePamphilis CW, Timko MP. Genome-wide
689 identification of MST, SUT and SWEET family sugar transporters in root parasitic
690 angiosperms and analysis of their expression during host parasitism. *BMC Plant Biol*
691 2019; **19**: 196.
- 692 40. Johnson DA, Hill JP, Thomas MA. The monosaccharide transporter gene family in land
693 plants is ancient and shows differential subfamily expression and expansion across
694 lineages. *BMC Evol Biol* 2006; **6**: 64.

- 695 41. Gerardy-Schahn R, Oelmann S, Bakker H. Nucleotide sugar transporters: Biological
696 and functional aspects. *Biochimie* 2001; **83**: 775–782.
- 697 42. Orellana A, Moraga C, Araya M, Moreno A. Overview of Nucleotide Sugar Transporter
698 Gene Family Functions Across Multiple Species. *J Mol Biol* 2016; **428**: 3150–3165.
- 699 43. Chen L-Q, Hou B-H, Lalonde S, Takanaga H, Hartung ML, Qu X-Q, et al. Sugar
700 transporters for intercellular exchange and nutrition of pathogens. *Nature* 2010; **468**:
701 527–532.
- 702 44. Maor-Landaw K, Eisenhut M, Tortorelli G, van de Meene A, Kurz S, Segal G, et al. A
703 candidate transporter allowing symbiotic dinoflagellates to feed their coral hosts. *ISME*
704 *Commun* 2023; **3**: 1–7.
- 705 45. An J, Zeng T, Ji C, de Graaf S, Zheng Z, Xiao TT, et al. A *Medicago truncatula* SWEET
706 transporter implicated in arbuscule maintenance during arbuscular mycorrhizal
707 symbiosis. *New Phytol* 2019; **224**: 396–408.
- 708 46. Manck-Götzenberger J, Requena N. Arbuscular mycorrhiza Symbiosis Induces a Major
709 Transcriptional Reprogramming of the Potato SWEET Sugar Transporter Family. *Front*
710 *Plant Sci* 2016; **7**.
- 711 47. Roy R, Reinders A, Ward J, McDonald T. Understanding transport processes in lichen,
712 *Azolla*–cyanobacteria, ectomycorrhiza, endomycorrhiza, and rhizobia–legume symbiotic
713 interactions. *F1000Research* 2020; **9**: 39.
- 714 48. Li X, Si W, Qin Q, Wu H, Jiang H. Deciphering evolutionary dynamics of SWEET genes
715 in diverse plant lineages. *Sci Rep* 2018; **8**: 13440.
- 716 49. Joshi S, Patil S, Hossain MA, Kumar V. *In-silico* identification and characterization of
717 sucrose transporter genes and their differential expression pattern under salinity stress
718 in *Glycine max*. *Plant Stress* 2024; **11**: 100314.
- 719 50. Liu H-T, Ji Y, Liu Y, Tian S-H, Gao Q-H, Zou X-H, et al. The sugar transporter system of
720 strawberry: genome-wide identification and expression correlation with fruit soluble
721 sugar-related traits in a *Fragaria* × *ananassa* germplasm collection. *Hortic Res* 2020; **7**:
722 132.

- 723 51. Weise SE, Liu T, Childs KL, Preiser AL, Katulski HM, Perrin-Porzondek C, et al.
724 Transcriptional Regulation of the Glucose-6-Phosphate/Phosphate Translocator 2 Is
725 Related to Carbon Exchange Across the Chloroplast Envelope. *Front Plant Sci* 2019;
726 **10**.
- 727 52. Teng GCY, Boo MV, Lam SH, Pang CZ, Chew SF, Ip YK. Molecular characterization
728 and light-dependent expression of glycerol facilitator (GlpF) in coccoid Symbiodiniaceae
729 dinoflagellates of the giant clam *Tridacna squamosa*. *Gene Rep* 2022; **27**: 101623.
- 730 53. Chin W-C, Orellana MV, Quesada I, Verdugo P. Secretion in Unicellular Marine
731 Phytoplankton: Demonstration of Regulated Exocytosis in *Phaeocystis globosa*. *Plant*
732 *Cell Physiol* 2004; **45**: 535–542.
- 733 54. González-Pech R A, Ragan M A & Chan C X. Signatures of adaptation and symbiosis
734 in genomes and transcriptomes of Symbiodinium. *Scientific Reports* 2017; **7**: 15021
- 735 55. Carradec Q, Pelletier E, Da Silva C, Alberti A, Seeleuthner Y, Blanc-Mathieu R, et al. A
736 global ocean atlas of eukaryotic genes. *Nat Commun* 2018; **9**: 373.
- 737 56. Zingone A, Chrétiennot-Dinet M-J, Lange M, Medlin L. Morphological and genetic
738 characterization of *Phaeocystis cordata* and *P. jahnii* (prymnesiophyceae), two new
739 species from the Mediterranean Sea. *J Phycol* 1999; **35**: 1322–1337.
- 740 57. Rousseau, V., Chrétiennot-Dinet, MJ., Jacobsen, A. et al. The life cycle of *Phaeocystis*:
741 state of knowledge and presumptive role in ecology. *Biogeochemistry* 2007; **83**, 29–47
- 742 58. Charrier G, Lacoite A, Améglio T. Dynamic Modeling of Carbon Metabolism During the
743 Dormant Period Accurately Predicts the Changes in Frost Hardiness in Walnut Trees
744 *Juglans regia* L. *Front Plant Sci* 2018; **9**.
- 745 59. Li Y, Xin G, Wei M, Shi Q, Yang F, Wang X. Carbohydrate accumulation and sucrose
746 metabolism responses in tomato seedling leaves when subjected to different light
747 qualities. *Sci Hortic* 2017; **225**: 490–497.
- 748 60. Shi Q, Chen C, He T, Fan J. Circadian rhythm promotes the biomass and amylose
749 hyperaccumulation by mixotrophic cultivation of marine microalga *Platymonas*
750 *helgolandica*. *Biotechnol Biofuels Bioprod* 2022; **15**: 75.

- 751 61. Grigoriev IV, Hayes RD, Calhoun S, Kamel B, Wang A, Ahrendt S, et al. PhycoCosm, a
752 comparative algal genomics resource. *Nucleic Acids Res* 2021; **49**: D1004–D1011.
- 753 62. Jones P, Binns D, Chang H-Y, Fraser M, Li W, McAnulla C, et al. InterProScan 5:
754 genome-scale protein function classification. *Bioinformatics* 2014; **30**: 1236–1240.
- 755 63. Saier MH Jr, Reddy VS, Moreno-Hagelsieb G, Hendargo KJ, Zhang Y, Iddamsetty V, et
756 al. The Transporter Classification Database (TCDB): 2021 update. *Nucleic Acids Res*
757 2021; **49**: D461–D467.
- 758 64. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer ELL, et al.
759 Pfam: The protein families database in 2021. *Nucleic Acids Res* 2020; **49**: D412–D419.
- 760 65. Cantalapiedra CP, Hernández-Plaza A, Letunic I, Bork P, Huerta-Cepas J. eggNOG-
761 mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at
762 the Metagenomic Scale. *Mol Biol Evol* 2021; **38**: 5825–5829.
- 763 66. Käll L, Krogh A, Sonnhammer ELL. A Combined Transmembrane Topology and Signal
764 Peptide Prediction Method. *J Mol Biol* 2004; **338**: 1027–1036.
- 765 67. Krogh A, Larsson B, von Heijne G, Sonnhammer ELL. Predicting transmembrane
766 protein topology with a hidden markov model: application to complete genomes1. *J Mol*
767 *Biol* 2001; **305**: 567–580.
- 768 68. Thumhuri V, Almagro Armenteros JJ, Johansen AR, Nielsen H, Winther O. DeepLoc
769 2.0: multi-label subcellular localization prediction using protein language models.
770 *Nucleic Acids Res* 2022; **50**: W228–W234.
- 771 69. Armenteros JJA, Salvatore M, Emanuelsson O, Winther O, Heijne G von, Elofsson A, et
772 al. Detecting sequence signals in targeting peptides using deep learning. *Life Sci*
773 *Alliance* 2019; **2**.
- 774 70. Gschloessl B, Guerneur Y, Cock JM. HECTAR: A method to predict subcellular
775 targeting in heterokonts. *BMC Bioinformatics* 2008; **9**: 393.
- 776 71. Horton P, Park K-J, Obayashi T, Fujita N, Harada H, Adams-Collier CJ, et al. WoLF
777 PSORT: protein localization predictor. *Nucleic Acids Res* 2007; **35**: W585–W587.

- 778 72. Jiang Y, Jiang L, Akhil CS, Wang D, Zhang Z, Zhang W, et al. MULocDeep web service
779 for protein localization prediction and visualization at subcellular and suborganellar
780 levels. *Nucleic Acids Res* 2023; **51**: W343–W349.
- 781 73. Fleet J, Ansari M, Pittman JK. Phylogenetic analysis and structural prediction reveal the
782 potential functional diversity between green algae SWEET transporters. *Front Plant Sci*
783 2022; **13**.
- 784 74. Picelli S, Faridani OR, Björklund ÅK, Winberg G, Sagasser S, Sandberg R. Full-length
785 RNA-seq from single cells using Smart-seq2. *Nat Protoc* 2014; **9**: 171–181.
- 786 75. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence
787 data. *Bioinformatics* 2014; **30**: 2114–2120.
- 788 76. Lu J, Salzberg SL. Removing contaminants from databases of draft genomes. *PLOS*
789 *Comput Biol* 2018; **14**: e1006277.
- 790 77. Bushmanova E, Antipov D, Lapidus A, Prjibelski AD. rnaSPAdes: a de novo
791 transcriptome assembler and its application to RNA-Seq data. *GigaScience* 2019; **8**:
792 giz100.
- 793 78. Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, et al. De novo
794 transcript sequence reconstruction from RNA-seq using the Trinity platform for
795 reference generation and analysis. *Nat Protoc* 2013; **8**: 1494–1512.
- 796 79. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO:
797 assessing genome assembly and annotation completeness with single-copy orthologs.
798 *Bioinformatics* 2015; **31**: 3210–3212.
- 799 80. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*
800 2012; **9**: 357–359.
- 801 81. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq
802 quantification. *Nat Biotechnol* 2016; **34**: 525–527.
- 803 82. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for
804 RNA-seq data with DESeq2. *Genome Biol* 2014; **15**: 550.

- 805 83. Quinn TP, Erb I, Gloor G, Notredame C, Richardson MF, Crowley TM. A field guide for
806 the compositional analysis of any-omics data. *GigaScience* 2019; **8**: giz107.
- 807 84. Li Z, Zhang Y, Li W, Irwin A J, Finkel Z V. Conservation and architecture of
808 housekeeping genes in the model marine diatom *Thalassiosira pseudonana*. *New*
809 *phytologist* 2022; 234: 1363-1376

810

811 **Figures & legends**

812 **Fig. 1. Genomic composition of sugar transporters of six haptophyte species.**

813 **A)** Gene composition and Pfam families of different sugar transporters found in the
814 genomes of six haptophytes species represented in a schematic phylogenetic tree
815 (*Diacronema lutheri*, *Emiliana huxleyi*, *Chrysochromulina tobinii*, *Phaeocystis*
816 *antarctica*, *Phaeocystis globosa*, *Phaeocystis cordata*). Eight Pfam families were found
817 and represented by different colors and the number of sugar transporter genes within
818 each Pfam is indicated. The genus *Phaeocystis* was found to contain between 156 and
819 184 sugar transporters out of 1066 to 1654 transporters in total. **B)** In *Phaeocystis*
820 *cordata*, the subcellular localization and number of sugars transporters are shown in
821 the schematic drawing of the microalga (prediction using a combination of *in silico*
822 tools, see methods and Table S1). **C)** For each Pfam, the proportion of sugar
823 transporters within each compartment of the cell are shown in the bar chart.

824 **Fig. 2. Expression of algal transporter genes between the free-living and symbiotic** 825 **stages of the microalga *Phaeocystis cordata*.**

826 **A)** Principal Component Analysis (PCA) of gene expression variance between free-
827 living (culture; light gray) and symbiotic *Phaeocystis cordata* (dark gray), including all
828 the expressed genes; X-axis represent variance according to sample types and y-axis
829 according to replicates within each sample type. **B)** PCA of expression variance of the
830 2,133 transporter genes in the free-living and symbiotic phases of *Phaeocystis cordata*.

831 **C)** Comparison of normalized read count values of the sugar and all others transporter
832 genes between the free-living and symbiotic stages of *Phaeocystis cordata* (Wilcoxon
833 rank sum test). **D)** Volcano plot showing the fold change of up- and down-regulated
834 transporter genes of symbiotic *Phaeocystis*, compared to the free-living stage,
835 including sugar transporters (blue circles) and other transporters (grey circles)
836 following DESeq2 analysis ($\log_2FC \geq 2$, $p\text{-adj} < 0.05$, see Table S3).

837

838 **Fig. 3. Expression of sugar transporter genes between the free-living and**
839 **symbiotic *Phaeocystis cordata* at the gene and Pfam level.**

840 **A)** Pairwise comparison of the normalized read counts of sugar transporter genes from
841 eight Pfams (shown by different colors) between free-living and symbiotic *Phaeocystis*
842 *cordata* (Wilcoxon rank sum test, sum of read counts > 10 over all replicates). Each dot
843 and line represents one sugar transporter gene. **B)** Distribution of fold changes for up-
844 and down-regulated sugar transporter genes in symbiotic *Phaeocystis* compared to
845 free-living ($\log_2FC > 2$, $p\text{-adj} < 0.05$). Each line corresponds to one sugar transporter
846 gene from a specific Pfam, which was found to be up- or down-regulated in a different
847 number of holobionts (Y axis, right, see also Table S3).

848

849 **Table 1. Differential expression of sugar transporter genes from different**
850 **Pfams in the symbiotic stage of *Phaeocystis cordata* compared to the free-**
851 **living one.** ($\log_2FC \geq 2$, $p\text{-adj} < 0.05$). Note that "neutral" indicates that the sugar
852 transporter gene is expressed in symbiosis, but not significantly down- or up-
853 regulated compared to the free-living stage.

854

855 **Fig. 4. Normalized *in situ* expression of sugar transporter genes of the microalga**
856 ***Phaeocystis cordata* in the Mediterranean Sea based on environmental**
857 **metatranscriptomic data.**

858 **A)** Geographic map showing the six different sampling stations from the *Tara Oceans*
859 expedition analyzed in this study. **B)** Comparison of the TPM (Transcript per Million)
860 values for sugar transporter genes between the small (0.8-5 μm) and large (180-2000
861 μm) size fractions for each Pfam (each point corresponds to the TPM value of one
862 sugar transporter gene). **C)** Comparison of TPM values of sugar transporter genes
863 between the small and large size fractions including those upregulated in the single-
864 holobiont transcriptomes (bold lines) (Fig. 3). In B and C, the TPM value of each sugar
865 transporter gene was normalized by the expression of housekeeping genes (see
866 Methods).

867

868 **Fig. 5. Temporal dynamics of the expression of the sugar transportome in the**
869 **symbiotic microalga *Phaeocystis cordata*.**

870 **A)** Venn diagram showing the number of sugar transporter genes expressed in one or
871 different conditions (morning, evening and dark-evening) (DESeq2 normalized read
872 counts >10 for one condition and <10 for the other two conditions). **B)** Comparison of
873 the normalized read counts of sugar transporter genes in different Pfams between
874 morning, evening and dark-evening; ns (non-significant) and * (significant < 0.05)
875 represent the Wilcoxon rank sum test significance for the comparisons between
876 morning/evening and evening/dark-evening. **C)** Comparison of mean values of
877 normalized read counts for the sugar transporter genes found upregulated in symbiosis
878 (Fig.3B) across the three conditions (morning, evening and dark-evening).

Figures of the manuscript

Transportome remodeling of a symbiotic microalga inside a planktonic host

Juery C, Auladell A, Füssy Z, Chevalier F, Yee DP, Pelletier E, Corre E, Allen, AE, Richter DJ,
Decelle, J

Caroline Juery: caroline.juery@cea.fr

Johan Decelle: johan.decelle@univ-grenoble-alpes.fr

This PDF file includes:

Fig. 1 to Fig. 5

Table1

Legends are provided at the end of the main text

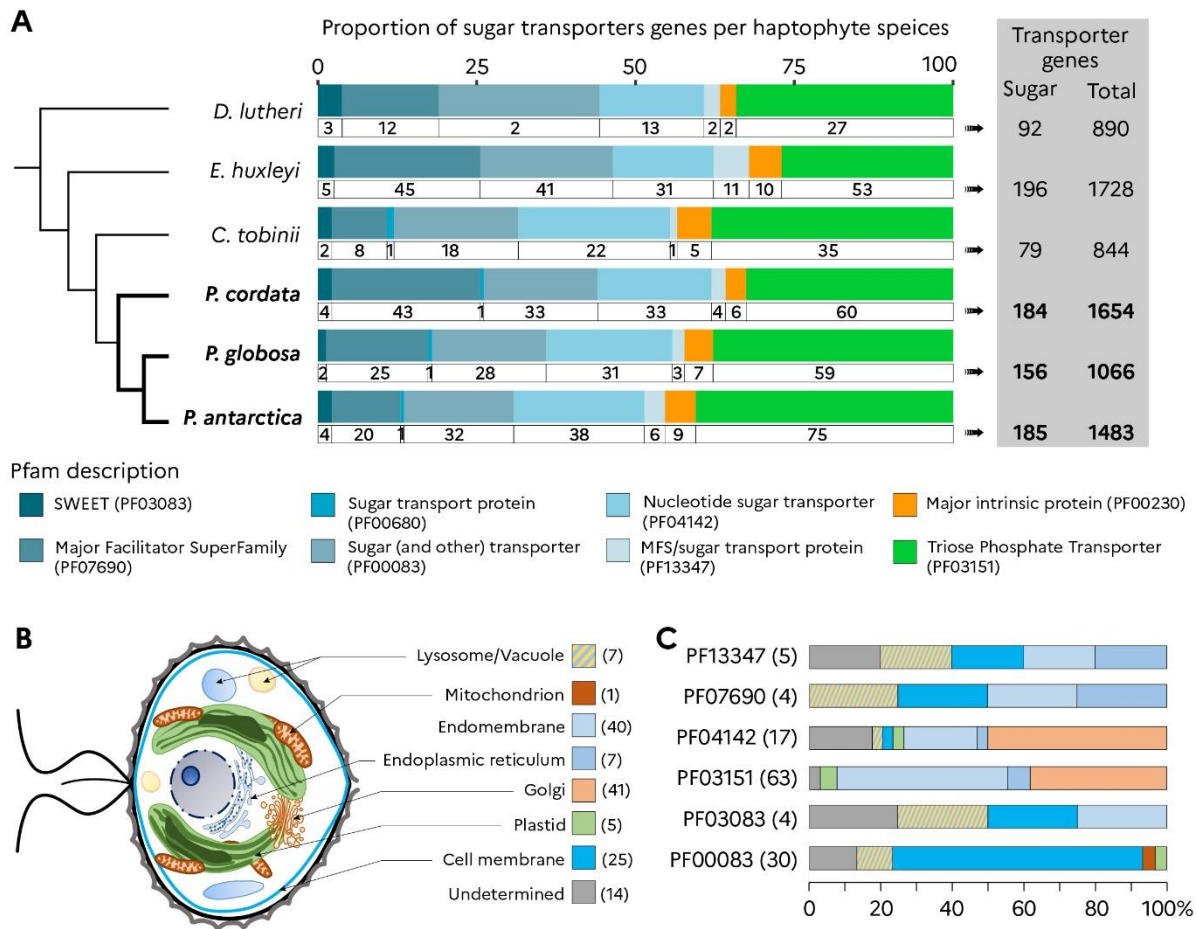


Figure 1

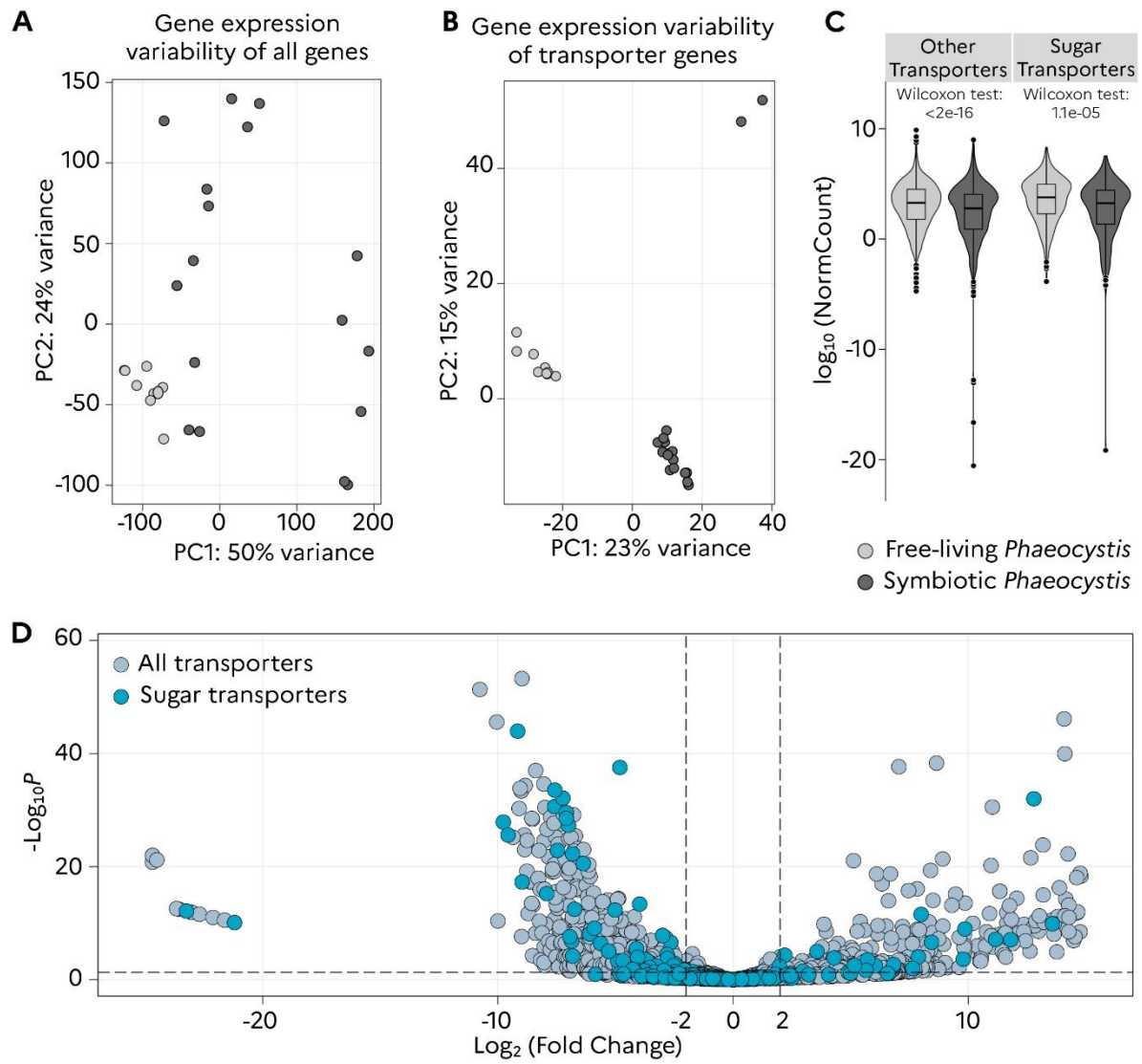


Figure 2

	Annotation		-2 < log2FC < 2 - padj 0.05			
	Pfam	Pfam_description	Down	Neutral	UP	Total
Sugar Transporters	PF00083	Sugar_(and_other)_transporter	13	28	2	43
	PF03083	Sugar_efflux_transporter_for_intercellular_exchange	1	5	0	6
	PF03151	Triose-phosphate_Transporter_family	19	56	10	85
	PF04142	Nucleotide-sugar_transporter	10	30	2	39
	PF07690	Major_Facilitator_Superfamily	2	9	2	13
	PF00230	Major_Intrinsic_protein	1	6	2	13
	PF13347	MFS/sugar_transport_protein	2	5	1	7
	PF00474	Sodium/glucose_cotransporter	0	1	0	1
	PF00892	GDP-fucose_transporter	0	1	0	1
	PF06800	Sugar_transport_protein	0	1	0	1
	PF08489	UDPxylose/SugarPhosphate_translocator	2	0	0	2
	-	L-arabinose	1	0	0	1
	-	GDP-fucose	1	6	0	7
	-	GDP-mannose	0	4	0	4
Total			52	152	19	223

Table 1

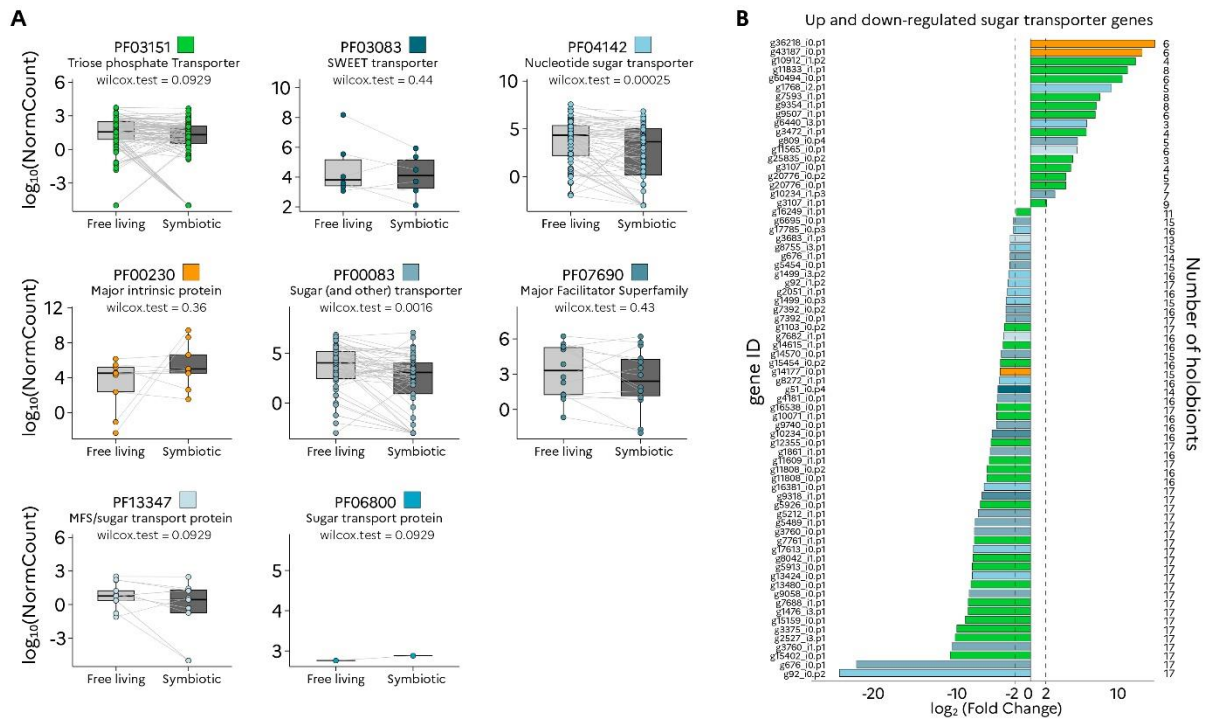


Figure 3

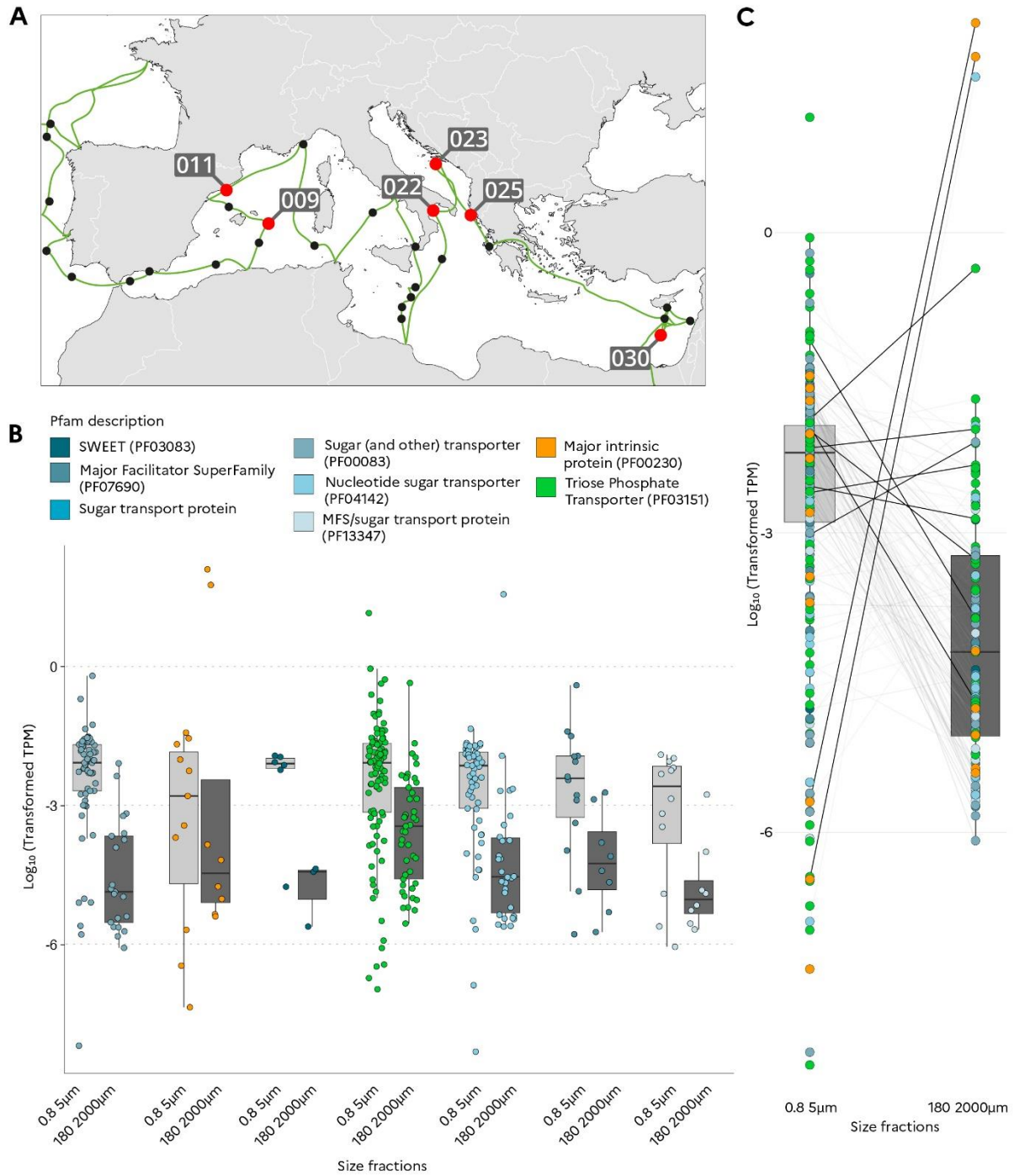


Figure 4

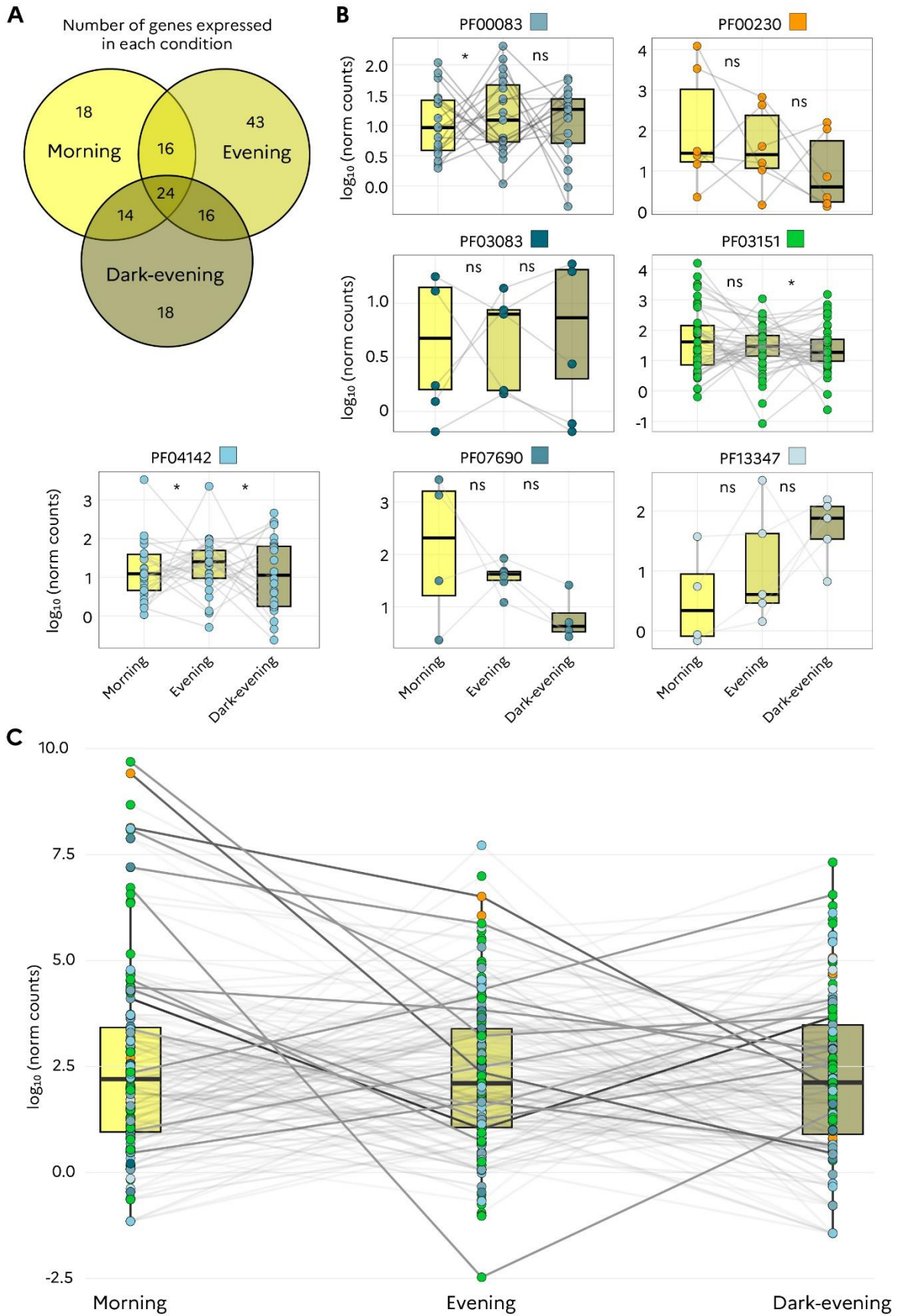


Figure 5