

**Small eukaryotic phytoplankton communities off Brazil are
dominated by symbioses between Haptophyta and nitrogen-fixing
cyanobacteria**

Supplementary material

Available at https://github.com/vaulot/Ribeiro_CARBOM_ISME_2018

Catherine Gérikas Ribeiro^{a,b,1}, Adriana Lopes dos Santos^{a,c1}, Dominique Marie^a, Frederico
Pereira Brandini^b, Daniel Vaulot^{a,2}

^a Sorbonne Université, CNRS, UMR7144, Station Biologique de Roscoff, Roscoff, France.

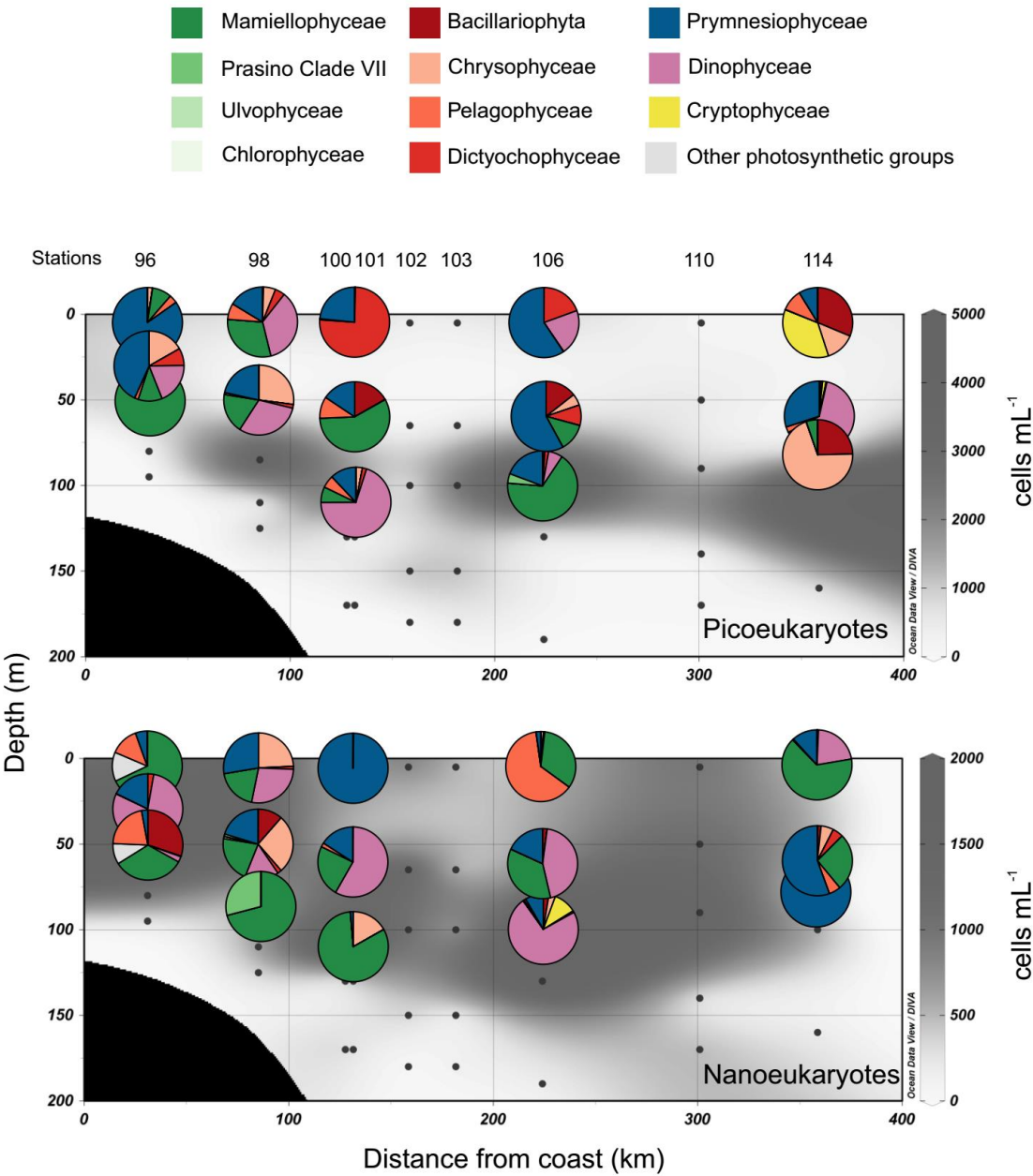
^b Departamento de Oceanografia Biológica, Instituto Oceanográfico, Universidade de São
Paulo, São Paulo, Brasil.

^c Centro de Genómica y Bioinformática, Facultad de Ciencias, Universidad Mayor. Camino La
Pirámide 5750, Huechuraba. Santiago, Chile.

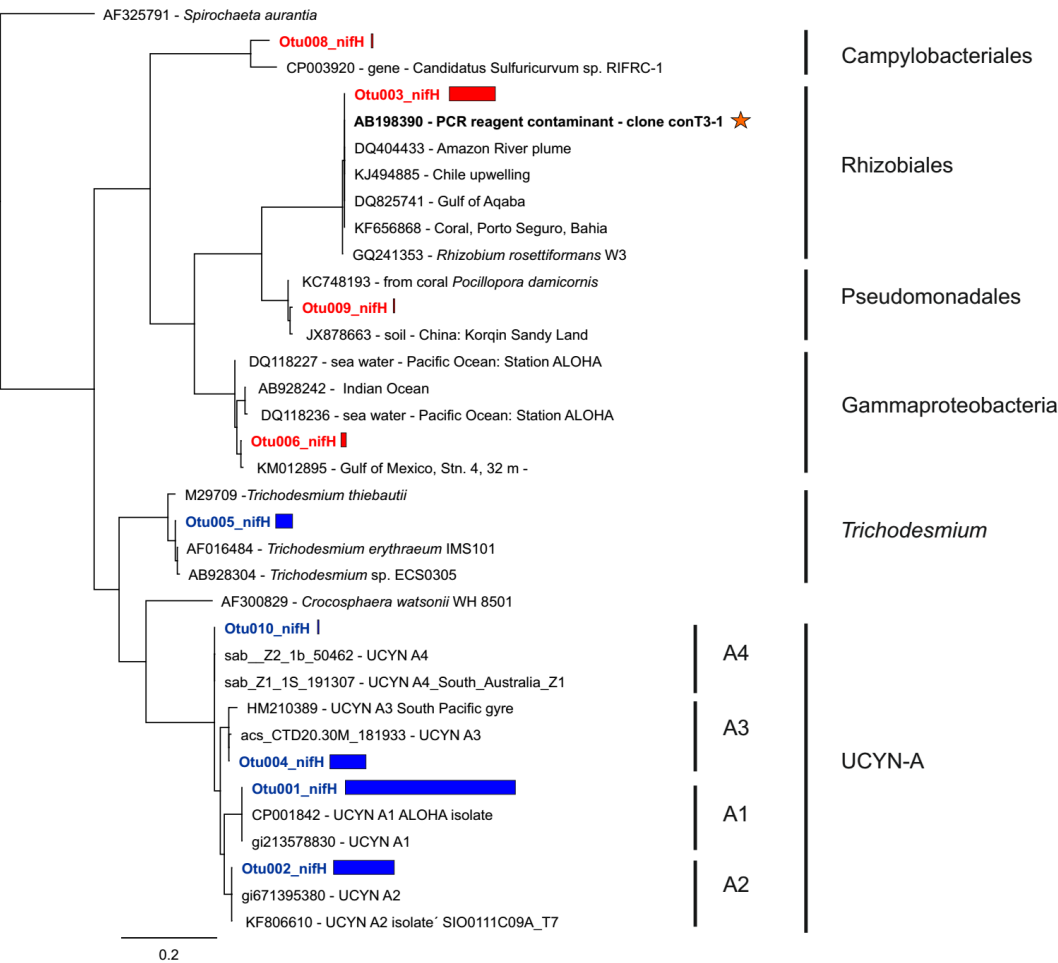
¹These two authors contributed equally to the paper:

²Corresponding author: vaulot@sb-roscoff.fr

20 **Supplementary Figures**



21
22 Supplementary Figure 1: Contribution of autotrophic groups along transect TR2. Top:
23 picoeukaryotes; Bottom: nanoeukaryotes. Concentration of each population determined by
24 flow cytometry is displayed in background (data from Gérikas Ribeiro *et al.*, 2016).



26

27 Supplementary Figure 2: Phylogenetic tree of major *nifH* OTUs (in blue and redcolor) built

28 using FastTree. The length of the solid bars corresponds to the number of reads obtained for

29 the different OTUs (see Supplementary Table 5). Only cyanobacteria OTUs labelled in blue

30 have been taken into account. The GenBank sequence in bold marked with a star corresponds

31 to a known contaminant from PCR reagents [50].

Supplementary Tables

Available at an Excel file at :

https://github.com/vaulot/Ribeiro_CARBOM_ISME_2018/blob/master/Ribeiro%20ISME%20Supplementary%20Tables%20version%203.0.xlsx

Supplementary Table 1: Position and date of stations sampled.

Transect	Station	Date	Latitude	Longitude
0	6	31/10/2013	-23.58	-41.78
	19	02/11/2013	-25.79	-40.36
	21	02/11/2013	-26.23	-40.09
	26	03/11/2013	-27.31	-39.38
1	81	13/11/2013	-27.42	-44.72
	85	13/11/2013	-26.80	-45.30
	86	13/11/2013	-26.33	-45.41
	87	13/11/2013	-26.22	-45.48
2	96	18/11/2013	-27.39	-47.82
	98	18/11/2013	-27.59	-47.39
	101	18/11/2013	-27.79	-46.96
	106	19/11/2013	-28.12	-46.17
	114	19/11/2013	-28.65	-44.99
	Bloom	18/11/2013	-27.80	-47.10

Supplementary Table 2: List of PCR primers used in this study for 18S rRNA and *nifH* amplification.

Gene	Round	Primer	Illumina tail	Sequence (5' - 3')
18S rRNA	1	63F	without	ACGCTTGTCTCAAAGATTA
18S rRNA	1	1818R	without	ACGGAAACCTTGTTACGA
18S rRNA	2	V4F_illum	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	CCAGCASCYGC GGTAATTCC
18S rRNA	2	V4R_illum	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	ACTTTCGTTCTTGATYRATGA
<i>nifH</i>	1	nifh3	without	ATRTTRTTNGCNGCRTA
<i>nifH</i>	1	nifh4	without	TTYTAYGGNAARGGNGG
<i>nifH</i>	2	nifh1_illum	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	TGYGAYCCNAARGCNGA
<i>nifH</i>	2	nifh2_illum	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	ADNGCCATCATYTCNCC

44 Supplementary Table 3: Number of sequences at each processing step.

	Total	18S	18S dereplicated	nifH	nifH dereplicated
Reads total	14 834 508				
Reads - Clean 1 - length>200	14 707 788				
Reads - Clean 2 Q >20 for 75% fo read	9 773 271				
Reads - after removing unpaired	7 917 631				
Contigs	7 917 631				
Contigs after removing ambuiguities	6 199 867				
pcr.seqs - pdiffs=2		3 948 941		1 490 889	
After unique		3 948 941	537 799	1 490 638	144 321
After remove singleton		3 515 396	104 254	1 380 363	34 046
After precluster pdiffs = 2		3 515 396	10 104	1 380 363	2 921
After removing chimeras		3 419 600	6 333	1 378 670	2 766
After removing control sequences		2 956 531	5 395	1 198 040	2 490
After removing sequences with less than 10 representative		2 944 506	871	1 191 857	296
OTUs at 98% similarity			287		22

45

46

47 Supplementary Table 4: List of major autotrophic 18S rRNA OTUs (representing at least 20% of sequences in at least one sample).

OTU	Number of reads	Division	Class	Genus or Clade	Species	BLAST best hit	Similarity	Best hit Description
Otu001	350 230	Chlorophyta	Mamiellophyceae	<i>Ostreococcus</i>	clade_B	AY425310	100.0%	<i>Ostreococcus</i> sp. RCC 143 small subunit ribosomal RNA gene, partial sequence
Otu002	337 596	Haptophyta	Prymnesiophyceae	UCYN A1 host		FJ537341	100.0%	Uncultured <i>Chrysochromulina</i> clone Biosope_T60.034 18S ribosomal RNA gene, partial sequence
Otu003	314 675	Chlorophyta	Mamiellophyceae	<i>Bathycoccus</i>	<i>prasinus</i>	FO082268	100.0%	<i>Bathycoccus prasinus</i> genomic : Chromosome_11
Otu004	148 588	Dinophyta	Dinophyceae	<i>Prorocentrum</i>	<i>sp.</i>	AY803742	100.0%	<i>Prorocentrum dentatum</i> isolate CCMP1517 small subunit ribosomal RNA gene, partial sequence
Otu005	83 434	Ochrophyta	Bacillariophyta	<i>Thalassiosira</i>	<i>sp.</i>	AB827486	100.0%	Uncultured diatom gene for 18S rRNA, partial sequence, clone: HP18D-43
Otu006	79 382	Ochrophyta	Bacillariophyta	<i>Pseudo-nitzschia</i>	<i>sp.</i>	KP709000	100.0%	<i>Pseudo-nitzschia kodamae</i> voucher PnPd31 18S ribosomal RNA gene, partial sequence
Otu007	77 275	Ochrophyta	Pelagophyceae	<i>Pelagomonas</i>	<i>calceolata</i>	AF363176	100.0%	Eukaryote marine clone ME1-27 clone ME1-27 18S ribosomal RNA gene, partial sequence
Otu008	73 570	Dinophyta	Dinophyceae			JX840913	100.0%	Uncultured dinoflagellate clone ARK_69 18S ribosomal RNA gene, partial sequence
Otu009	69 360	Haptophyta	Prymnesiophyceae	<i>Chrysochromulina</i>	<i>sp.</i>	EU500075	100.0%	Uncultured eukaryote clone hotxp4d1 18S ribosomal RNA gene, partial sequence
Otu011	66 030	Ochrophyta	Chrysophyceae	Clade C		KT893827	100.0%	<i>Spumella</i> sp. GW3 18S ribosomal RNA gene, partial sequence
Otu012	60 498	Dinophyta	Dinophyceae	<i>Gonyaulax</i>	<i>polygramma</i>	EU792964	100.0%	Uncultured dinoflagellate clone PROSOPE.C3-5m.55 18S ribosomal RNA gene, partial sequence
Otu014	47 176	Ochrophyta	Chrysophyceae	Clade G		JX291679	100.0%	Uncultured eukaryote clone 7656BH1002_SP6 18S ribosomal RNA gene, partial sequence
Otu015	46 716	Dinophyta	Dinophyceae			EU500117	99.2%	Uncultured eukaryote clone hotxp1f10 18S ribosomal RNA gene, partial sequence
Otu018	32 238	Ochrophyta	Dictyochophyceae	Pedinellales		JX291680	100.0%	Uncultured eukaryote clone 7656BH1003_SP6 18S ribosomal RNA gene, partial sequence
Otu020	30 200	Haptophyta	Prymnesiophyceae	<i>Braarudosphaera</i>	<i>bigelowii</i>	AB250784	100.0%	<i>Braarudosphaera bigelowii</i> gene for 18S rRNA, partial sequence, isolate: TP05-6-a
Otu022	29 363	Haptophyta	Prymnesiophyceae	<i>Syracosphaera</i>	<i>pulchra</i>	AM490987	100.0%	<i>Syracosphaera pulchra</i> partial 18S rRNA gene, strain ALGO GK 17
Otu023	29 297	Ochrophyta	Bacillariophyta	Bacillariophyceae		KU743708	100.0%	Uncultured stramenopile clone 10B3D21 small subunit ribosomal RNA gene, partial sequence
Otu025	27 592	Chlorophyta	Mamiellophyceae	<i>Micromonas</i>	<i>commoda</i>	CP001575	99.7%	<i>Micromonas</i> sp. RCC299 chromosome 8, complete sequence
Otu026	27 168	Ochrophyta	Bacillariophyta	<i>Cylindrotheca</i>	<i>closterium</i>	AY866417	100.0%	<i>Cylindrotheca closterium</i> strain MGB0401 18S ribosomal RNA gene, partial sequence
Otu027	26 619	Dinophyta	Dinophyceae	<i>Karlodinium</i>	<i>sp.</i>	AB827455	98.9%	Uncultured dinoflagellate gene for 18S rRNA, partial sequence, clone: HP18D-12
Otu028	24 686	Haptophyta	Prymnesiophyceae	<i>Emiliana</i>	<i>huxleyi</i>	KT861255	99.7%	<i>Emiliana huxleyi</i> strain RCC909 18S ribosomal RNA gene, partial sequence
Otu030	23 299	Cryptophyta	Cryptophyceae	<i>Teleaulax</i>	<i>sp.</i>	KT815862	100.0%	Uncultured eukaryote clone 44c_22195 18S ribosomal RNA gene, partial sequence
Otu032	20 607	Chlorophyta	Prasinophytes Clade A4			KF615770	99.7%	Prasinophyceae sp. CCMP1998 18S ribosomal RNA gene, partial sequence
Otu033	20 430	Chlorophyta	Ulvophyceae	<i>Ulva</i>	<i>sp.</i>	AB425964	99.2%	<i>Ulva fasciata</i> gene for 18S ribosomal RNA, partial sequence, isolate:
Otu034	20 273	Dinophyta	Dinophyceae	Dinophyceae		FJ914420	99.2%	Uncultured marine dinoflagellate clone B19 18S ribosomal RNA gene, partial sequence
Otu035	19 931	Ochrophyta	Chrysophyceae	Clade G		JX291795	100.0%	Uncultured eukaryote clone 7656BH979_SP6 18S ribosomal RNA gene, partial sequence
Otu038	16 274	Ochrophyta	Chrysophyceae	Clade G		KJ759278	100.0%	Uncultured eukaryote clone SGYO1048 18S ribosomal RNA gene, partial sequence
Otu039	15 821	Haptophyta	Prymnesiophyceae	<i>Algirosphaera</i>	<i>robusta</i>	AM490985	100.0%	<i>Algirosphaera robusta</i> partial 18S rRNA gene, strain ALGO Am 24
Otu040	15 101	Dinophyta	Dinophyceae			JX291868	99.5%	Uncultured eukaryote clone 7657BH1618_SP6 18S ribosomal RNA gene, partial sequence
Otu041	14 875	Ochrophyta	Chrysophyceae	Clade I		EF695207	100.0%	Uncultured eukaryote isolate hotp1g9 18S ribosomal RNA gene, partial sequence
Otu042	13 963	Dinophyta	Dinophyceae			EU500202	98.9%	Uncultured eukaryote clone hotpCh1 18S ribosomal RNA gene, partial sequence
Otu044	11 744	Chlorophyta	Mamiellophyceae	<i>Micromonas</i>	<i>bravo</i>	AY425316	100.0%	<i>Micromonas pusilla</i> small subunit ribosomal RNA gene, partial sequence
Otu072	4 877	Haptophyta	Prymnesiophyceae	Prymnesiales		KF129663	99.5%	Uncultured eukaryote clone ST3500.080 18S ribosomal RNA gene, partial sequence

48

49

50 Supplementary Table 5: List of major cyanobacteria *nifH* OTUs.

OTU	Number of reads	Class	Genus or Clade	BLAST best hit	Similarity	Best hit Description
Otu001	591 206	Cyanobacteria	UCYN_A1	HQ611363	100.00%	Uncultured bacterium clone 46401A11 dinitrogenase reductase (<i>nifH</i>) mRNA, partial cds
Otu002	211 526	Cyanobacteria	UCYN_A2	KF806610	100.00%	Candidatus Atelocyanobacterium thalassa isolate SIO0111C09A_T7 dinitrogenase reductase (<i>nifH</i>) gene, partial cds
Otu003	159 676	Rhizobiales	<i>Rhizobium</i>	FN665898	100.00%	Uncultured bacterium partial mRNA for dinitrogenase reductase (<i>nifH</i> gene), clone Mex69-290_B07
Otu004	124 990	Cyanobacteria	UCYN_A3	HM210392	100.00%	Uncultured bacterium clone HH1879 NifH (<i>nifH</i>) mRNA, partial cds
Otu005	57 926	Cyanobacteria	<i>Trichodesmium</i>	KX520212	100.00%	Uncultured bacterium clone 1103_21228_174051_CTD4_200 dinitrogenase reductase (<i>nifH</i>) gene, partial cds
Otu006	17 919	Gammaproteobacteria		KM012895	99.70%	Uncultured bacterium clone M1507A01 dinitrogenase reductase (<i>nifH</i>) gene, partial cds
Otu008	6 454	Campylobacteriales	<i>Sulfuricurvum</i>	KR132029	92.20%	Uncultured nitrogen-fixing bacterium clone C21 dinitrogenase reductase (<i>nifH</i>) gene, partial cds
Otu009	4 974	Pseudomonadales	<i>Azotobacter</i>	JX878659	99.10%	Uncultured bacterium clone 1 nitrogenase reductase (<i>nifH</i>) gene, partial cds
Otu010	4 661	Cyanobacteria	UCYN_A4	LC013598	100.00%	Uncultured marine bacterium <i>nifH</i> gene for nitrogenase, partial cds, clone: KK-13-1_ON5_1

51

52

Supplementary Material

This material is available at https://github.com/vaulot/Ribeiro_CARBOM_ISME_2018

Supplementary Material 1: Mothur processing scripts

(https://github.com/vaulot/Ribeiro_CARBOM_ISME_2018/tree/master/scripts_mothur).

Supplementary Material 2: R processing script with all necessary input files

(https://github.com/vaulot/Ribeiro_CARBOM_ISME_2018/tree/master/scripts_R).

Supplementary Material 3: Contingency table for 18S rRNA OTUs

(CARBOM 18S otus 0.02 abundance.xlsx)

Supplementary Material 4: Contingency table for *nifH* OTUs

(CARBOM nifH otus 0.02abundance.xlsx).

Supplementary Material 5: 18S rRNA OTUs sequences (CARBOM 18S otus 0.02.fasta)

Supplementary Material 6: *nifH* rRNA OTUs sequences (CARBOM nifH otus 0.02.fasta)

Supplementary Material 7: Environmental variables measured during the CARBOM V cruise

(scripts_R /carbom_envdata.txt)