Scientific Reports—Article

Title:

Genomic divergence within non-photosynthetic cyanobacterial endosymbionts in rhopalodiacean diatoms

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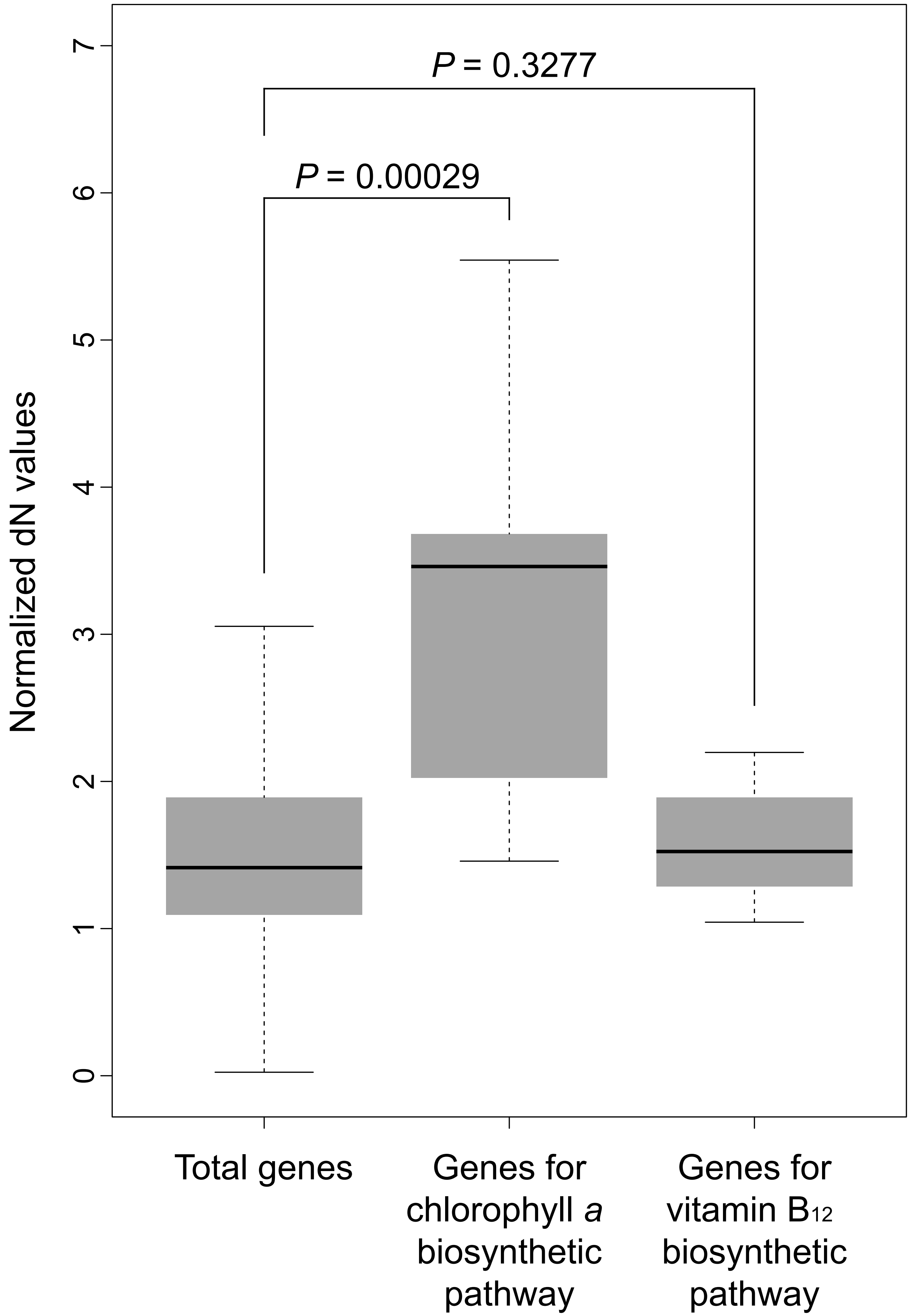
Supplementary Figure Legends

Figure S1. Normalized rates of non-synonymous substitution for total gene set and genes for the chlorophyll *a* and vitamin B_{12} biosynthetic pathways. Each boxplot represents the distribution of non-synonymous substitution rates (dN values) on protein-coding genes in the *Rg*SB genome, which were normalized by that of counterpart genes from *Cyanothece* spp. genomes (see material and method section in the main text). The difference in the normalized dN values between the total gene set and the Chl-*a* biosynthetic genes appeared to be significant (the Wilcoxon rank-sum test, $P = 0.00029$). On the other hand, no significant difference in the dN value distribution was observed in the same test considering the genes for vitamin B_{12} biosynthesis (the Wilcoxon rank-sum test, $P = 0.3277$). Outliers were not displayed in this graph.

Figure S2. Sequence comparison of the methionine synthase from the *Rg*SB, *Et*SB and free-living cyanobacteria. (a) Multiple-alignment of the amino acid sequences. (b) Pairwise identity matrix of sequences displayed in (a). The values indicate percentages of amino acid identity.

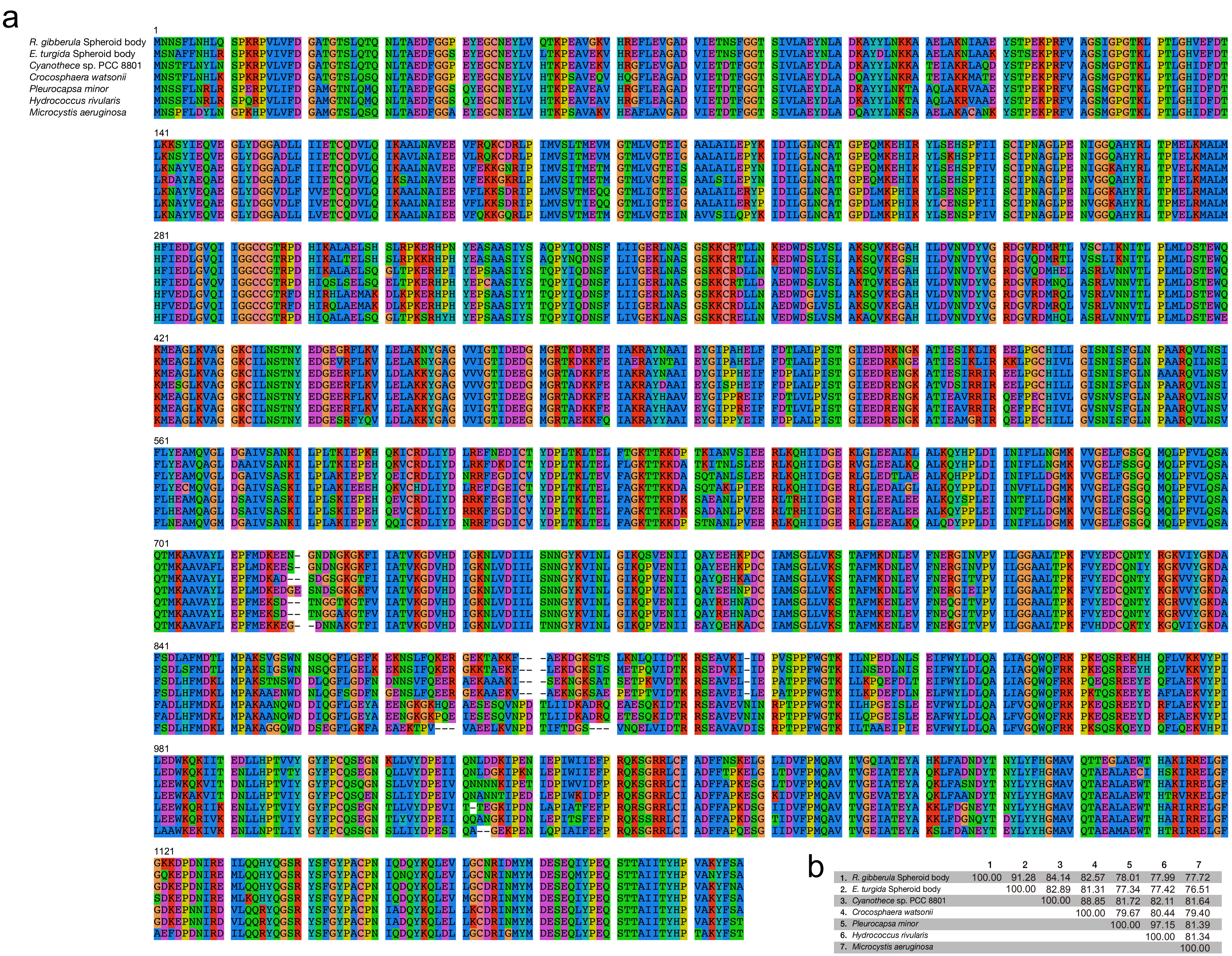
Figure S3. Phylogenetic tree topologies used in CODEML analyses.

Figure S1



P = 0.3277
P = 0.00029
Normalized dN values
0
1
2
3
4
5
6
7
Total genes
Genes for chlorophyll a biosynthetic pathway
Genes for vitamin B12 biosynthetic pathway

Figure S2



a

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                              1
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E. turgida Spheroid body      MSNAFFNHLR SPKRPVIVFD GATGTSLQTQ NLTAEDFGGS EYEGCNEYLV LTKPEAVEKV HREFLEAGAD VIETNSFGGT SIVLAEYNLA DKAYDLNKKA AELAKNLAAK YSTSEKPRFV AGSIGPGTKL PTLGHVEFDT
Cyanothece sp. PCC 8801       MNSTFLNYLN SPKRPVLVFD GATGTSLQTQ NLTAEDFGGP EYEGCNEYLV HTKPEAVEKV HRGFLEVGAD VIETDTFGGT SIVLAEYDLA DKAYYLNKKA TEIAKRLAQD YSTPEKPRFV AGSMGPGTKL PTLGHIDFDT
Crocosphaera watsonii         MNSTFLNHLK SPKRPVLVFD GATGTSLQSQ NLTAEDFGGP EYEGCNEYLV HTKPSAVEQV HQGFLEAGAD VIETDTFGGT SIVLAEYDLA DQAYYLNKRA TEIAKKMATE YSTPEKPRFV AGSMGPGTKL PTLGHIDFDT
Pleurocapsa minor             MNSSFLNRLR SPERPVLIFD GAMGTNLQMQ NLTAEDFGGS QYEGCNEYLV HTKPEAVEAV HRGFLEAGAD VIETDTFGGT SIVLAEYDLA DKAYYLNKTA AQLAKRVAAE YSTPEKPRFV AGSMGPGTKL PTLGHIDFDT
Hydrococcus rivularis         MNSSFLNRLR SPQRPVLIFD GAMGTNLQMQ NLTAEDFGGS QYEGCNEYLV HTKPEAVEAV HRGFLEAGAD VIETDTFGGT SIVLAEYDLA DKAYYLNKTA AQLAKRVAAE YSTPEKPRFV AGSMGPGTKL PTLGHIDFDT
Microcystis aeruginosa        MNSPFLDYLN GPKHPVLVFD GAMGTSLQSQ NLTAEDFGGA EYEGCNEYLV HTKPSAVAKV HEAFLAVGAD VIETDTFGGT SIVLAEYDLA DQAYYLNKSA AELAKACANK YSTPEKPRFV AGSMGPGTKL PTLGHIDFDT

141
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LKNSYIEQVE GLYDGGADLL IIETCQDVLQ IKAVLNAIEE VLRQKCDRLP IMVSITMEVM GTMLVGTEIG AALAILEPYK IDILGLNCAT GPEQMKEHIR YLSKHSPFII SCIPNAGLPE NIGGQAHYRL TPIELKMALM
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LKNAYVEQAE GLYDGGVDLF VVETCQDVLQ IKAALNAIEE VFLKKSDRIP LMVSVTMEQQ GTMLIGTEIG AALAILERYP IDILGLNCAT GPDLMKPHIR YLSENSPFII SCIPNAGLPE NLGGKAHYRL TPMELRMALM
LKNAYVEQAE GLYDGGVDLF IVETCQDVLQ IKAALNAIEE VFLKKSDRIP LMVSVTIEQQ GTMLIGTEIG AALAILERYP IDILGLNCAT GPDLMKPHIR YLCENSPFII SCIPNAGLPE NVGGKAHYRL TPMELRMALM
LKNAYVEQVE GLYDGGADLL LVETCQDVLQ IKAALNAIEE VFQKKGQRLP LMVSVTMETM GTMLVGTEIN AVVSILQPYK IDILGLNCAT GPDLMKPHIK YLSENSPFIV SCIPNAGLPE NVGGQAHYRL TPVELKMALM

281
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HFIEDLGVQI IGGCCGTRPD HIKALTELSH SLRPKKRHPH YEASAASIYS AQPYNQDNSF LIIGEKLNAS GSKKCRTLLN KEDWDSLISL AKSQVKEGSH ILDVNVDYVG RDGVQDMRTL VSSLIKNITL PLMLDSTEWQ
HFIEDLGVQI IGGCCGTRPD HIKALAELSQ GLTPKERHPI YEPSAASIYS TQPYIQDNSF LIVGERLNAS GSKKCRDLLN VEDWDSLVSL AKSQVKEGAH ILDVNVDYVG RDGVQDMHEL ASRLVNNVTL PLMLDSTEWQ
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421
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561
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FLYEAVQAGL DAAIVSANKI LPLTKIEPEH QKVCRDLIYD LRKFDKDICT YDPLTKLTEL FLGKTTKKDA TKITNLSLEE RLKQHIIDGE RVGLEEALKQ ALKQYHPLDI INIFLLNGMK VVGELFSSGQ MQLPFVLQSA
FLYEAMQVGL DGAIVSASKI LPLAKIEPEY QEICRDLIYD NRRFEGDICV YDPLTKLTEV FAGKTTKKDA SQTANLSLEE RLKQHIIDGE RLGLEDTLAE ALKQHPPLDI INIFLLDGMK VVGELFGSGQ MQLPFVLQSA
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701
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QTMKAAVAFL EPLMDKEES- GNDNGKGKFI IATVKGDVHD IGKNLVDIIL SNNGYKVINL GIKQPVENII QAYEEHKPDC IAMSGLLVKS TAFMKDNLEV FNERGINVPV ILGGAALTPK FVYEDCQNIY KGKVVYGKDA
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QTMKAAVAFL EPFMEKKEG- -DNNAKGTFI IATVKGDVHD IGKNLVDIIL TNNGYRVINL GIKQPVENII EAYQEHKADC IAMSGLLVKS TAFMKENLEV FNEKGITVPV ILGGAALTPK FVHDDCQKTY KGQVIYGKDA

841
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FSDLSFMDTL MPAKSIGSWN NSQGFLGELK EKNSIFQKER GKKTAEKF-- -LEKDGKSIS METPQVIDTK RSEDVKI-ID PVSPPFWGTK ILNSEDLNIS EIFWYLDLQA LIAGQWQFRK PKEQSREEYH QFLVKKVYPI
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981
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LEEWKQKVIT DNLLHPTVIY GYFPCQSQGN SLLVYDPEIV QNNNNKIPET IDPIWVIDFP RQKSGRRLCI ADFFAPKESG LTDVFPMQAV TVGEIATEYA QKLFASNDYT NYLYYHGMAV QTAEALAEWT HAKIRRELGF
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1121
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GQKEPDNIRE MLQQHYQGSR YSFGYPACPN IQNQYKQLEV LGCNRINMYM DESEQMYPEQ STTAIITYHP VANYFSA
GDKEPDNIRE MLQQHYQGSR YSFGYPACPN IQDQYKQLEI LGCDRINMYM DESEQIYPEQ STTAIITYHP VAKYFNA
SDKEPDNIRE MLKQHYQGSR YSFGYPACPN IQDQYKQLEV MGCDRINMYM DESEQIYPEQ STTAIITYHP VAKYFSA
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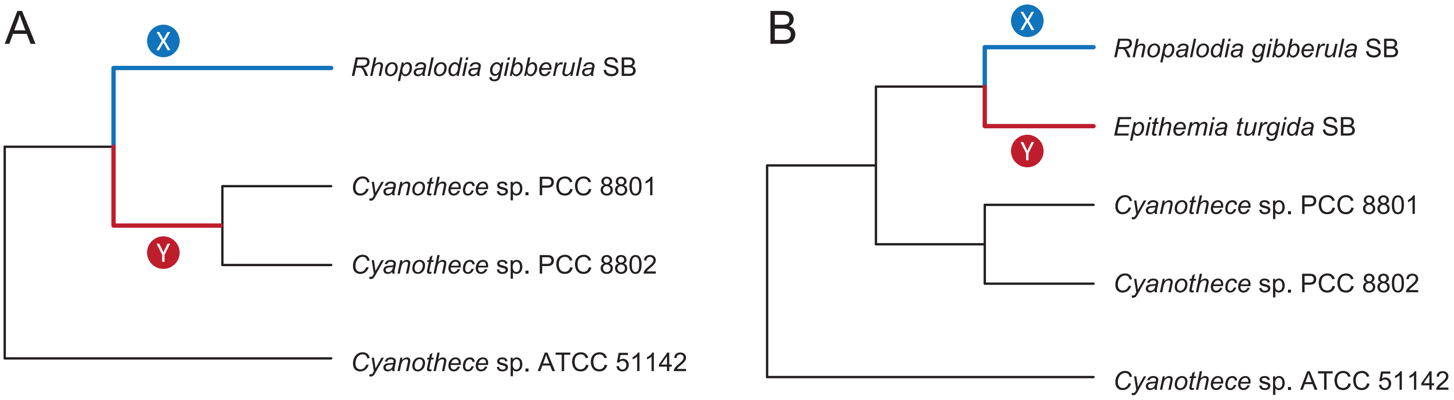
GDKEPNNIRD VLQQRYQGSR YSFGYPACPN IADQYKQLEL LKCDRINMYM DESEQLYPEQ STTAIITYHP VAKYFST
AEFDPDNIRD ILQQRYQGSR YSFGYPACPN IQDQYKQLDL LGCDRIGMYM DESEQLYPEQ STTAIITYHP TAKYFST
```

b

	1	2	3	4	5	6	7
1. *R. gibberula* Spheroid body	100.00	91.28	84.14	82.57	78.01	77.99	77.72
2. *E. turgida* Spheroid body		100.00	82.89	81.31	77.34	77.42	76.51
3. *Cyanothece* sp. PCC 8801			100.00	88.85	81.72	82.11	81.64
4. *Crocosphaera watsonii*				100.00	79.67	80.44	79.40
5. *Pleurocapsa minor*					100.00	97.15	81.39
6. *Hydrococcus rivularis*						100.00	81.34
7. *Microcystis aeruginosa*							100.00

Figure S3


A
X
Rhopalodia gibberula SB
Cyanothece sp. PCC 8801
Y
Cyanothece sp. PCC 8802
Cyanothece sp. ATCC 51142
B
X
Rhopalodia gibberula SB
Epithemia turgida SB
Y
Cyanothece sp. PCC 8801
Cyanothece sp. PCC 8802
Cyanothece sp. ATCC 51142