Supplementary figures



Figure S1: Example of determination of the phycobilisome (PBS) breaking temperature (T_{PBS}) of *Synechococcus* sp. MVIR-16-2 grown at 16°C. The logarithm of the phycocyanin to phycoerythrin fluorescence emission ratio (PE:PC) is related to rod phycobiliprotein coupling. The temperature at which PBS breaks down (T_{PBS}), determined in a similar way as Arrhenius calculation (Dalhoff et al. 1991, Stillman et al. 1996) constitutes a proxy for assessing phycobilisome thermostability.

References

- Dahlhoff E, O'Brien J, Somero GN, & Vetter RD (1991) Temperature effects on mitochondria from hydrothermal vent invertebrates: evidence for adaptation to elevated and variable habitat temperatures. *Physiological Zoology* 64(6):1490-1508.
- Stillman J & Somero G (1996) Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus Petrolisthes): correlation of physiology, biochemistry and morphology with vertical distribution. *The Journal of Expe Biol*199(8):1845-1855.



Figure S2: Absorption spectra of phycoerythrin II (PE; **A**) phycoerythrin I (**B**) and phycocyanin (PC; **C**) from the subtropical clade II *Synechococcus* sp. strain M16.1 measured over a thermal denaturation curve. PCB, Phycocyanobilin. PEB; Phycoerythrobilin; PUB: Phycourobilin.



Figure S3: Temperature-induced decay of phycobiliprotein absorbance for six marine *Synechococcus* strains. Phycoerythrin II (orange), phycoerythrin I (pink) and phycocyanin (purple) absorption at 545 nm for the phycoerythrins and 620 nm for phycocyanin along thermal denaturation curves in marine *Synechococcus* spp. M16.1 (**A**), RS9907 (**B**), WH7803 (**C**), ROS8604 (**D**), MVIR-16-2 (**E**) and MVIR-18-1 (**F**). Unfolding curves allowed the calculation of $T_{50\%}$, defined as the temperature at which the phycobiliprotein has lost half of its absorption capacities. Standard deviations are calculated from the mean of three replicates.



Figure S4: Variation of phycoerythrin II (orange) and I (pink) and phycocyanin (purple) fluorescence emission properties, upon excitation at 545 nm for phycoerythrins and 620 nm for phycocyanin, along thermal denaturation curves in marine *Synechococcus* spp. M16.1 (**A**), RS9907 (**B**), WH7803 (**C**), ROS8604 (**D**), MVIR-16-2 (**E**) and MVIR-18-1 (**F**). The extinction fluorescence slope for each phycobiliprotein is mentioned in inserts. Standard deviations are calculated from the mean of three replicates.



Figure S5: Mid-unfolding temperature ($T_{50\%}$) of phycoerythrin (PE) II, phycoerythrin I and phycocyanin (PC) as a function of isolation latitude of the six marine *Synechococcus* strains studied in this paper. For each phycobiliprotein and each strain, standard deviations are calculated from the mean based on three replicates.

	RpcA					
	-	1 10	20	30	40	50
	ROS8604	MKTPLTEAVAAADSQGF	RFLSNTEVQAA	SGRFNRAKAS	LEAAK <mark>G</mark> LTAK	ADS
	MVIR-18-1	MKTPLTEAVAAADSQGF	RFLSNTEVQAAS	SGRFNRAQAS	LEAAK <mark>G</mark> LTAK	ADS
Ι	WH8016	MKTPLTEAVAAADSQGF	RFLSNTEVQAA	SGRFNRAKAS	LEAAK <mark>G</mark> LTAK	ADS
	WH8020	MKTPLTEAVAAADSQGF	RFLSNTEVQAA	SGRFNRAKAS	LEAAK <mark>G</mark> LTAK	ADS
	CC9311	MKTPLTEAVAAADSQGF	RFLSNTEVQAAS	SGRFNRAKAS	ldaak <mark>g</mark> ltak	ADA
T T T	BL107	MKTPLTEAVAAADSQGF	RFLSNTEVQAA	SGRFNRAKAS	leaak <mark>g</mark> ltsk	AEA
$\perp \lor$	CC9902	MKTPLTEAVAAADSQGF	RELSNTEVQAA	SGRFNRAKAS	leaak <mark>g</mark> ltsk	ADA
	M16.1	MKTPLTEAVAAADSOGF	RFLSNTEVOAA	SGRFNRAKAS	leaak <mark>a</mark> ltsk	ADS
	TAK9802	MKTPLTEAVAAADSOGF	RFLSNTEVOAA	SGRFNRAKAS	leaakaltsk	ADS
	RS9902	MKTPLTEAVAAADSOGF	RFLSNTEVOAA	SGRFNRAKAS	leaak <mark>a</mark> ltsk	ADS
	RS9907	MKTPLTEAVAAADSOGF	RFLSNTEVOAA	SGRFNRAKAS	LEAAKALTSK	ADS
ΙI	A15-44	MKTPLTEAVAAADSOGF	RFLSNTEIOGAI	FGRFNRAKAA	LEAAKALTTK	ADT
	CC9605	MKTPLTEAVAAADSOGE	FLSNTEVOAA	SGRENRAKAS	LEAAKALTGK	ADS
	A15-62	MKTPLTEAVAAADSOGE	FLSNTEVOAA	SGRENRAKAS	LEAAKALTSK	ADS
	WH8109	MKTPLTEAVAAADSOGE	FLSNTEVOAA	SGRENRAKAS	LEAAKALTSK	ADS
	PROS-U-1	MKTPLTEAVAAADSOGE	FLSNTEVOAA	SGRENRAKAS	LEAAKALTSK	ADS
	WH8102	MKTPLTFAVAAADSOGG	FLONTEVOID	SCRENBARAS	LEVAKVILLOK	ADG
	WH0102		VELONTEVQAA.	SGRENRARAS	TEVVKVILCK	ADS
ттт	N10105	MKTDI TEAVAAADSQGF		COFNDAVAG	TEANKALISK	ADS ADA
т т т	ALJ-20 DOUM119	MKTPLTEAVAAADSQGP	T LONTEVQAA	CDENDARAS	LEAARALISK	ADA
	DC0015	MKIPLIEAVAAADSQGP		SGRENRARAS	LEAARALISE	ADS
	R59915	MKIPLIEAVAAADSQGF		SGRENRARAS	LEAARALISE	ADS
V	WH/OUS	MKIPLIEAVAAADSQGF	ELSNIEVQAA	SGRENRAAAS	LEAAKALISK	ADS
	BMK-MC-1	MKIPLIEAVAAADSQGF	(FLSNTEVQGA)	E GRENRASAS	LEAAKALISK	ADS
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	RpcB	1 10	20	30	40	50
	RpcB Ros8604	1 10 MFDAFTKVVAOADARGO	20 FISSSEIDALS	30 Samvsdsdkri	40 LDSVSRLSSN	50 ASTB
	RpcB ROS8604 MVIR-18-1	1 10 MFDAFTKVVAQADARGQ MFDAFTKVVAQADARGO	20 FISSSEIDALS FISANEIDALA	30 Samvsdsdkri Aamvsgsnkri	40 LDSVSRLSSNA LDAVSRISNNA	50 ASTB ASTB
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I IV II	RpcB ROS8604 MVIR-18-1 WH8016 WH8020 CC9311 BL107 CC9902 M16.1 TAK9802 RS9902 RS9907 A15-44 CC9605 A15-62 WH8109 PROS-U-1 WH8102 WH8103 A15-28 BOUM118	1 10 MFDAFTKVVAQADARGQ	20 FISSSEIDALS FISANEIDALA FISSSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA	30 SAMVSDSDKRI AMVSGSNKRI AMVSGSNKRI AMVSGSNKRI AMVSDSNKRI AAVVSDSNKRI AAVVSDSNKRI AAVVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI	40 LDSVSRLSSN LDAVSRISNN LDAVSRISNN LDAVSRISNN LDAVSRISN LDAVSRISSN LDAVRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN	50 ASTB ASTB ASTB ASTB ASTB ASTB ASTB ASSB ASS
I IV II	RpcB Ros8604 MVIR-18-1 WH8016 WH8020 CC9311 BL107 CC9902 M16.1 TAK9802 RS9902 RS9907 A15-44 CC9605 A15-62 WH8109 PROS-U-1 WH8102 WH8103 A15-28 BOUM118 RS9915	1 10 MFDAFTKVVAQADARGQ	20 FISSSEIDALS FISANEIDALA FISSSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA	30 SAMVSDSDKRI AMVSGSNKRI AMVSGSNKRI AMVSGSNKRI AAVVSDSNKRI AAVVSDSNKRI AAVVSDSNKRI AAVVSDSNKRI AAMVSDSNKRI AAMVSDSNKRI AAMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI	40 LDSVSRLSSN LDAVSRISNN LDAVSRISNN LDAVSRISNN LDAVSRISN LDAVSRISSN LDAVSRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN	50 ASTB ASTB ASTB ASTB ASTB ASTB ASTB ASSB ASS
I IV II III	RpcB Ros8604 MVIR-18-1 WH8016 WH8020 CC9311 BL107 CC9902 M16.1 TAK9802 RS9907 A15-44 CC9605 A15-62 WH8109 PROS-U-1 WH8102 WH8103 A15-28 BOUM118 RS9915 WH7803	1 10 MFDAFTKVVAQADARGQ	20 FISSSEIDALS FISANEIDALA FISSSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA FISTSEIDALA	30 SAMVSDSDKRI AMVSGSNKRI AMVSGSNKRI AMVSGSNKRI AAVVSDSNKRI AAVVSDSNKRI AAVVSDSNKRI AAVVSDSNKRI AAVVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI AMVSDSNKRI	40 LDSVSRLSSN LDAVSRISNN LDAVSRISNN LDAVSRISNN LDAVSRISN LDAVSRISN LDAVSRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN LDAVNRISSN	50 ASTB ASTB ASTB ASTB ASTB ASTB ASTB ASSB ASS

Figure S6: Amino acid sequences alignment of phycocyanin α and β -subunits showing the substitution of alanine in the warm-adapted clades II and III to glycine in the cold-adapted clades I and IV on residue 43 of RpcA (A) and asparagine to serine on residue 42 of RpcB (B). Substitutions are indicated by yellow highlighting, and clades are differently colored : clade I, light blue ; clade II, red ; clade III, orange ; clade IV, dark blue.

 Table S1: Phycobiliprotein sequences used for comparative amino acid and structural analyses. The database compiled encompasses the sequences of the two subunits of
21 phycocyanin (RpcA, RpcB), 21 phycoerythrin I (CpeA, CpeB) and 20 phycoerythrin II (MpeA, MpeB). Accession numbers of the amino acid sequences are reported, and previously unpublished sequences are in bold.

Strain name	Clade	Pigment Type ¹	RCC ² number	lso. lat.	Iso. long.	lso. temp.	RpcA	RpcB	СреА	СреВ	МреА	MpeB
CC9311	I.	3dA	1086	31.9	-12.46	16.59	ABI45378	ABI47294	ABI45327	ABI46374	ABI45816	ABI45939
WH8016	I.	3aA	2535	41.53	-70.67	17.83	EHA59372	EHA59373	EHA59379	EHA59378	EHA59384	EHA59385
ROS8604	I.	За	2380	48.43	-3.59	12.81	AHF23553	AHF23552	AHF23616	AHF23615	KT955729	KT955719
MVIR-18-1	I.	3aA	2385	61.00	1.59	13.98	AGX70106	AHF23550	AHF23614	AHF23613	AGX70093	AGX70092
WH8020	I.	3dA	2437	38.41	-69.19	16.89	AHF23549	AHF23548	AHF23612	AHF23611	AAA27333	AAA273332
M16.1	П	3a	791	27.42	-91.18	24.15	AHF23571	AHF23570	AHF23634	AHF23633	KT955731	KT955721
A15-44	Ш	2	2527	21.41	-17.50	22.61	AHF23587	AHF23586	AHF23650	AHF23649	n.a.	n.a.
RS9902	Ш	3c	2376	29.28	34.55	21.07	AHF23579	AHF23578	AHF23642	AHF23641	KT955728	KT955718
RS9907	Ш	3a	2382	29.28	34.55	28.99	AHF23573	AHF23572	AHF23636	AHF23635	KT955727	KT955717
TAK9802	Ш	3a	2528	-14.30	-145.2	29.81	AHF23575	AHF23574	AHF23638	AHF23637	KT955725	KT955715
WH8109	Ш	3bB	2033	39.29	-70.28	21.60	AHF62838	AHF62839	AHF62845	AHF62844	AHF62860	AHF62861
A15-62	Ш	3dB	2374	17.37	-20.57	26.02	AHF23577	AHF23576	AHF23640	AHF23639	KT955733	KT955723
CC9605	Ш	3c	753	30.41	-123.98	18.02	ABB34196	ABB34197	ABB34210	ABB34211	ABB34210	ABB34211
PROS-U-1	Ш	3dB	2369	30.80	-10.30	21.51	AHF23581	AHF23580	AHF23644	AHF23643	KT955730	KT955720
BOUM118	Ш	3c	2379	33.38	32.38	25.20	AHF23563	AHF23562	AHF23626	AHF23625	KT955732	KT955722
RS9915	Ш	3dB	2553	29.28	34.55	26.98	AHF23567	AHF23566	AHF23630	AHF23629	KT955726	KT955716
WH8102	Ш	3c	539	22.29	-65.36	25.78	CAE08538	CAE08537	CAE08531	CAE08532	CAE08524	CAE08523
WH8103	Ш	3cA	29	28.30	-67.23	25.45	AHF23565	AHF23564	AHF23628	AHF23627	CRY93003	CRY93002
A15-28	Ш	3c	2556	31.15	-20.43	25.15	AHF23569	AHF23568	AHF23632	AHF23631	КТ955734	KT955724
CC9902	IV	3dA	2673	32.90	-117.25	15.56	ABB26866	ABB26865	ABB26851	ABB26850	ABB26851	ABB26850
BL107	IV	3dA	515	41.43	3.33	13.89	EAU70259	EAU70260	EAU70266	EAU70265	EAU70274	EAU70275

n.a.: not applicable (subunit not present in this strain).

¹ According to 4, 5 ² RCC: Roscoff Culture Collection (<u>http://roscoff-culture-collection.org/</u>)