1 Supplementary results

2 Identification of novel bacteriohopanepolyols

Six novel BHPs with the m/z 762.5, 656.5, 771.6, 748.5, 638.5, and 743.6 Da were tentatively 3 identified using MS² spectra and molecular formulas derived from accurate masses (Table S2). All 4 5 novel BHPs contained amino or nitro groups and yielded the characteristic fragment at m/z 191.2 6 fragment, indicating ring cleavage of hopanoids not methylated at C-2 or C-3. BHP-762.5 $(C_{43}H_{72}NO_{10}^+, [M+H]^+)$ was tentatively identified as tetra-functionalized, containing three 7 8 hydroxyl groups indicated by consecutive neutral loss of 42/60 (acetylated hydroxyl groups, COCH₂/CH₃COOH) and one nitro group (neutral loss of 47). The molecular formula indicates a 9 lack of acetylation at one functional group, which is also observed in other BHPs such as 10 adenosylhopane, and which could be related to steric effects. BHP-656.6 ($C_{40}H_{66}NO_6^+$, $[M+H]^+$) 11 was tentatively identified as a tetra-functionalized BHP bearing an amino group within a lactone 12 13 ring, indicated by neutral losses of 17 (NH₃) and 44 (CO₂; Crotti et al., 2005), respectively. The fragmentation pattern of BHP-771.6 ($C_{46}H_{79}N_2O_7^+$, $[M+H]^+$) suggests a tetra-functionalized BHP 14 containing two hydroxyl groups (neutral loss of 42/60), an amino group (neutral loss of 59; Talbot 15 16 et al., 2001), and an ether-bound aminopropanol group indicated by sequential neutral loss of 59 (acetamido; C_2H_5NO) plus 58 (C_3H_6O). BHP-748.5 ($C_{42}H_{70}NO_{10}^+$) produced a dominant in-source 17 fragment in MS¹ mode at m/z 688.5 (neutral loss of 60). Fragmentation of 688.5 yielded two neutral 18 losses of 60 as well as neutral losses of 75 (CHNO₃) and 93 (CH₃NO₄). Fragmentation spectra thus 19 indicate that BHP-748.5 is tetra-functionalized and contains three hydroxyl groups and a fourth 20 hydroxyl group that is bound to a nitrogen-containing functional group. Based on the neutral loss 21 of 93, we speculate that the nitrogen-containing functional group could be nitroformic acid or 22 nitroperoxymethane. BHP-638.5 could not be identified with confidence due to its low abundance 23

24 and consequent noisy spectra. However, accurate mass analysis suggests a molecular formula of $C_{40}H_{64}NO_5^+$ ([M+H]⁺) and thus the presence of a nitrogen-containing functional group. BHP-743.6 25 produced a dominant MS¹ in-source fragment at m/z 684.5 (neutral loss of 59). Accurate mass 26 analysis suggests a molecular formula of $C_{44}H_{75}N_2O_7^+$ ([M+H]⁺), suggesting the presence of two 27 nitrogen-containing functional groups. Fragmentation of m/z 684.5 yielded three neutral losses of 28 60 and a neutral loss of 31 (methylamine, CH₅N), suggesting that BHP-743.6 is penta-29 functionalized. Presence of one neutral loss of 59 but lack of a second neutral loss of 59 indicates 30 that the second nitrogen-containing group is not acetylated. This suggests that the second nitrogen-31 32 containing group consists of an amino nitrogen bound to the hopanoid backbone and bound to a methyl group that prevents acetylation. BHP-743.6 thus represents a penta-functionalized 33 hopanoid containing three hydroxyl groups, an amino group, and a methylamine group. 34

36 Supplementary Datafile captions

Supplementary Datafile S1 (Supplied as supplementary .xlsx file). Occurrence (+) and absence (-)
of hopanoid biosynthesis genes in AOB and NOB genomes from cultures and environmental
samples (metagenome-assembled genomes and single cell genomes) as well as from closely
related non-nitrifying taxa. Accession numbers, ecological metadata (taxonomy, habitat, Cfixation pathway, metabolism), and references to original work describing genomic data are given.

Supplementary Datafile S2 (Supplied as supplementary xlsx file). Sheet 1: Average relative 42 abundance (%, +/- standard deviation, values below 0.01 rounded to 0.01) of 43 bacteriohopanepolyols in triplicate cultures of nitrite-oxidizing bacteria grown under different 44 conditions. b.d., below detection. Standard deviations omitted for experiments performed without 45 replicates (Nitrobacter vulgaris and Nitrococcus mobilis with pseudovitamin B₁₂). Sheet 2: 46 Average relative abundance (%, +/- standard deviation, values below 0.01 rounded to 0.01) of 47 bacteriohopanepolyols (BHPs) and diploptene/methylated diploptene in tripliate cultures of nitrite-48 49 oxidizing bacteria grown under different conditions. b.d., not detected. Standard deviations omitted for experiments performed without replicates (Nitrobacter vulgaris and Nitrococcus 50 *mobilis* with pseudovitamin B_{12}). Sheet 3: Average abundance of total hopanoids (BHPs + 2-Me 51 Diploptene + 2-Me BHPs) in triplicate cultures normalized to mmol NO_2^- oxidized. Standard 52 deviations omitted for experiments performed without three replicates (Nitrobacter vulgaris and 53 54 Nitrococcus mobilis with pseudovitamin B_{12} as well as NO₂-limited and O₂-limited experiments

55 with *Nitrococcus mobilis* and *Nitrospina gracilis*).

56 Supplementary tables and figures

57 Table S1. Growth characteristics of nitrite-oxidizing bacteria in batch cultures (n.a., not available).

Studio	Crowth condition	NO ₂ -oxidation
Strain	Growth condition	rate (mM d ⁻¹)
Nitrospira marina 295	Autotrophic, early stationary	0.22 ± 0.05
Nitrospira marina 295	Autotrophic, late stationary	n.a.
Nitrospira marina 295	Autotrophic, methionine, early stationary	0.11 ± 0.07
Nitrospira marina 295	Autotrophic, vitamin B12, early stationary	0.3 ± 0.14
Nitrospira marina 295	Mixotrophic, early stationary	0.58 ± 0.11
Nitrospira lenta BS10	Autotrophic, early stationary	n.a.
Nitrospira defluvii A17	Autotrophic, early stationary	n.a.
Nitrospira moscoviensis M-1	Autotrophic, early stationary	n.a.
Nitrospina gracilis Nb-3/211	Autotrophic, early stationary	0.74 ± 0.01
Nitrospina gracilis Nb-3/211	Autotrophic, methionine, early stationary	0.74 ± 0.01
Nitrospina gracilis Nb-3/211	Autotrophic, vitamin B12, early stationary	0.74 ± 0.02
Nitrococcus mobilis 231	Autotrophic, early stationary	1.16 ± 0.26
Nitrococcus mobilis 231	Autotrophic, late stationary	n.a.
Nitrococcus mobilis 231	Autotrophic, methionine, early stationary	1.2 ± 0.01
Nitrococcus mobilis 231	Autotrophic, vitamin B12, early stationary	1.25 ± 0.1
Nitrobacter vulgaris AB1	Autotrophic, mid-growth phase	1.89 ± 0.07
Nitrobacter vulgaris AB1	Autotrophic, early stationary	2.14 ± 0.1
Nitrobacter vulgaris AB1	Autotrophic, methionine, early stationary	2.34 ± 0.08
Nitrobacter vulgaris AB1	Autotrophic, vitamin B12, early stationary	2.62 ± 0.05
Nitrobacter vulgaris AB1	Autotrophic, vitamin B12/methionine/light, early stationary	2.66 ± 0.22
Nitrobacter vulgaris AB1	Mixotrophic, early stationary	n.a.
Nitrobacter vulgaris AB1	Heterotrophic aerobic, early stationary	n.a.
Nitrobacter vulgaris AB1	Heterotrophic anaerobic, early stationary	n.a.

58

60 Table S2. Growth characteristics of *Nitrobacter vulgaris* AB1 in chemostat experiments under NO₂⁻-limited

61	and O ₂ -limited conditi	ons (average of tripl	icates $\pm 1\sigma$ standa	ard deviation	; b.d., be	low detec	ction $\sim < 0.1$	ppm).
	-			~	~			

	NO ₂ ⁻ -limited	O ₂ -limited
O ₂ dissolved, inflow (ppm)	8.6 ± 0.2	8.6 ± 0.2
O ₂ dissolved, reactor (ppm)	6 ± 0.2	b.d.
NO ₂ ⁻ dissolved, inflow (mM)	10	10
NO ₂ ⁻ dissolved, reactor (mM)	0.009 ± 0.002	5.82 ± 0.14
Medium volume, reactor (L)	2.0	2.0
Medium inflow rate (ml min ⁻¹)	0.443	0.443
Growth rate (h ⁻¹)	0.013	0.013
Doubling time (h)	52.2	52.2

- 64 Table S3. Growth characteristics of Nitrospina gracilis Nb-211 and Nitrococcus mobilis Nb-231 in
- 65 chemostat experiments under NO₂⁻-limited and O₂-limited conditions (average of quadruplicates $\pm 1\sigma$
- 66 standard deviation; b.d., below detection).

	N. gr	acilis	N. ma	obilis
	NO2 ⁻ -limited	O ₂ -limited	NO2 ⁻ -limited	O ₂ -limited
NO ₂ ⁻ dissolved, inflow (mM)	2.0	2.0	2.0	2.0
NO ₂ ⁻ dissolved, outflow (mM)	b.d.	1.1	b.d.	1.1
Medium volume, reactor (L)	2.0	2.0	2.0	2.0
Medium in-/outflow rate (ml min ⁻¹)	0.37	0.37	0.37	0.37
Growth rate (h ⁻¹)	0.011	0.011	0.011	0.011
Doubling time (h)	62.5	62.5	62.5	62.5
Cell concentration (cells ml ⁻¹)	$1.04\pm0.21\times10^{8}$	$0.86\pm0.14 imes10^8$	$1.83\pm0.46\times10^{8}$	$2.76\pm0.62\times10^8$
Specific NO ₂ ⁻ ox. rate (fmol cell ⁻¹ d ⁻¹)	20.0 ± 4.8	10.1 ± 1.7	11.5 ± 2.9	3.2 ± 0.7
Growth yield (cells mol NO ₂ ⁻ ox. ⁻¹)	$5.19 \pm 1.05 \times 10^{13}$	$1.01 \pm 1.59 \times 10^{14}$	$9.13 \pm 2.28 \times 10^{13}$	$3.20 \pm 0.71 \times 10^{14}$

69 Table S4. Observed mass, predicted mass, predicted sum formula, measurement error inferred from

70 predicted and observed masses, and double-bond equivalents (Dbl. eq.) of base peak (underlined) and major

71 fragment ions in MS² spectra of novel BHPs from *Nitrobacter vulgaris* AB1 and *Nitrococcus mobilis* 231.

Compound	I Ion	Observed mass (Da)	Predicted mass (Da)	Predicted formula	Error (ppm)	Error (mDa)	Dbl. eq.
BHP-762.5	$[M+H]^+$	762.5101	762.5151	$C_{43}H_{72}NO_{10}{}^{+}$	6.5	5.0	8.5
	$[M-CH_3COOH]^+$	702.4886	702.4939	$C_{41}H_{68}NO_8^+$	7.6	5.3	8.5
	[M-CH ₃ COOH-CH ₂ CO] ⁺	660.4766	660.4834	$C_{39}H_{66}NO_7^+$	10.3	6.8	7.5
	[M-2xCH ₃ COOH-CH ₂ CO] ⁺	600.4557	600.4622	$C_{37}H_{62}NO_5^+$	10.9	6.6	7.5
	[M-2xCH ₃ COOH-CH ₂ CO-NO ₂ H] ⁺	553.4653	553.4615	$C_{37}H_{61}O_{3}^{+}$	-6.8	-3.8	7.5
	[M-3xCH ₃ COOH-CH ₂ CO-NO ₂ H] ⁺	493.4438	493.4404	$C_{35}H_{57}O^+$	-6.9	-3.4	7.5
	$[M-3xCH_3COOH-CH_2CO-NO_2H-H_2O]^+$	475.4339	475.4298	$C_{35}H_{55}^{+}$	-8.6	-4.1	8.5
BHP-656.5	5 [M+H] ⁺	<u>656.4950</u>	656.4885	$C_{40}H_{66}NO_{6}^{+}$	-10	-6.5	8.5
	[M-CO ₂] ⁺	612.5040	612.4986	$C_{39}H_{66}NO_4^+$	-8.8	-5.4	7.5
	[M-CO ₂ -CH ₃ COOH] ⁺	552.4824	552.4775	$C_{37}H_{62}NO_2^+$	-8.9	-4.9	7.5
	[M-CO ₂ -2xCH ₃ COOH] ⁺	492.4600	492.4564	$C_{35}H_{58}N^{\scriptscriptstyle +}$	-7.4	-3.6	7.5
	$[M-CO_2-2xCH_3COOH-NH_3]^+$	475.4363	475.4298	$C_{35}H_{55}^+$	-13.6	-6.5	8.5
BHP-771.6	5 [M+H] ⁺	771.5944	771.5882	$C_{46}H_{79}N_2O_7^+$	-8.1	-6.2	8.5
	[M-CH ₃ CONH ₂] ⁺	712.5506	712.5511	$C_{44}H_{74}NO_{6}^{+}$	0.7	0.5	8.5
	[M-CH ₃ CONH ₂ -CH ₃ CHCH ₂ O] ⁺	654.5143	654.5092	$C_{41}H_{68}NO_5^+$	-7.8	-5.1	8.5
	[M-CH ₃ CONH ₂ -CH ₃ CHCH ₂ O-CH ₃ COOH] ⁺	594.4902	594.4881	$C_{39}H_{64}NO_{3}^{+}$	-3.6	-2.1	8.5
	$[M-CH_3CONH_2-CH_3CHCH_2O-2xCH_3COOH]^+$	534.4707	534.4669	$C_{37}H_{60}NO^{\scriptscriptstyle +}$	-7	-3.8	8.5
	[M-CH ₃ CONH ₂ -CH ₃ CHCH ₂ O-2xCH ₃ COOH- CH ₃ CONH ₂] ⁺	475.4352	475.4298	$C_{35}H_{55}^{+}$	-11.3	-5.4	8.5
BHP-748.5	5 [M+H] ⁺	748.4982	748.4994	$C_{42}H_{70}NO_{10}^{+}$	1.6	1.2	8.5
	$[M-CH_3COOH]^+$	688.4800	688.4783	$C_{40}H_{66}NO_8^+$	-2.5	-1.7	8.5
	[M-2xCH ₃ COOH] ⁺	628.4577	628.4572	$C_{38}H_{62}NO_{6}^{+}$	-0.9	-0.5	8.5
	[M-3xCH ₃ COOH] ⁺	568.4321	568.4360	$C_{36}H_{58}NO_4^+$	6.9	3.9	8.5
	[M-3xCH ₃ COOH-CHNO ₃] ⁺	493.446	493.4404	$C_{35}H_{57}O^+$	-11.4	-5.6	7.5
	[M-3xCH ₃ COOH-CH ₃ NO ₄] ⁺	475.4311	475.4298	$C_{35}H_{55}^{+}$	-2.7	-1.3	8.5
BHP-638.5	5 [M+H] ⁺	<u>638.4823</u>	638.4779	$C_{40}H_{64}NO_5^+$	-6.9	-4.4	9.5
BHP-743.6	5 [M+H] ⁺	743.5651	743.5569	$C_{44}H_{75}N_2O_7^+$	-11.1	-8.2	8.5
	[M-CH ₃ CONH ₂] ⁺	<u>684.5213</u>	684.5198	$C_{42}H_{70}NO_{6}^{+}$	-2.2	-1.5	8.5
	[M-CH ₃ CONH ₂ -CH ₃ COOH] ⁺	624.5041	624.4986	$C_{40}H_{66}NO_4^+$	-8.7	-5.5	8.5
	[M-CH ₃ CONH ₂ -2xCH ₃ COOH] ⁺	564.4848	564.4775	$C_{38}H_{62}NO_2^+$	-12.9	-7.3	8.5
	$[M-CH_3CONH_2-3xCH_3COOH]^+$	504.4550	504.4564	$C_{36}H_{58}N^+$	2.7	1.4	8.5
	[M-CH ₃ CONH ₂ -3xCH ₃ COOH-CH ₃ N] ⁺	473.4252	473.4142	$C_{35}H_{53}^{+}$	-23.3	-11	9.5

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- 73

Table S5. Absence (-) of marker genes for the aerobic (*cobF*, *cobG*) and anaerobic (*cbiD*, *cbiG*)
cobalamin biosynthesis pathways as well as genes for cobalamin-dependent (*metH*) and
cobalamin-independent (*metE*) methionine synthase in seven species of nitrite-oxidizing bacteria,
indicating lack of cobalamin biosynthesis in four of these organisms. Presence of marker genes
was determined through blast (Altschul et al., 1990) analysis using protein query sequences from *Salmonella typhimurium* LT2, *Escherichia coli* K12, *Pseudomonas denitrificans*, and

Halobacterium salinarum.

	cobF	cobG	cbiD	cbiG	metE	metH
Nitrospira marina 295	-	-	-	-	-	+
Nitrospina gracilis 3/211	-	-	-	-	-	+
Nitrobacter vulgaris AB1	-	-	-	-	+	+
Nitrococcus mobilis 231	-	-	-	-	+	+
Nitrospira defluvii A17	-	-	+	+	-	+
Nitrospira lenta BS10	-	-	+	+	-	+
Nitrospira moscoviensis M-1	-	-	+	+	-	+





Phase 2: Oxygen-limited (nitrite-replete)



- **Fig. S1.** The chemostat setup used to grow *N. gracilis, N. mobilis and N. vulgaris* in continuous
- culture under nitrite-limited and oxygen-limited conditions.



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Fig. S2. Phylogenetic tree of hopanoid C-2 (*hpnP*) and C-3 (*hpnR*) methylase homologues in
bacteria. Colors indicate nitrite-oxidizing bacteria (NOB; red), ammonia-oxidizing bacteria (AOB;
cyan), and complete ammonia-oxidizing bacteria (comammox; purple). Strains where presence of
C-2 or C-3 methyl hopanoids was tested and detected are highlighted in bold (based on this study
and Rohmer et al., 1984; Welander et al., 2010; Welander and Summons, 2012; Kool et al., 2014;
Sinninghe Damsté et al., 2017) and strains that were not tested are set in normal font. Circles
indicate branches with >85% support based on 500 bootstrap analyses. The scale bar represents 1

94 substitution per amino acid.

	Rhodopseudomonas palustris TIE-1	DV	A F M P P Q G L	LV I AA	YLPI	D E <mark>W</mark> S	VRF	DEN	I RA <mark>A</mark>	TAD	F AW.	AD <mark>A</mark> I	VFV	S GMH I Q	RQQ	MNDIC	CR <mark>RA</mark> F	DFDL	. PVA	LGGPSV	ACPD	YYPI	FDYL	H∀GI	ELGDAT	DQ	I AK L	THD	-VT	R P K F	R <mark>QV</mark> V	FTTE	DRL
	Methylorubrum extorquens PA1	GV	(AFMPPQG <mark>L</mark>	L L <mark>I A</mark> A	YM P	etwe	CRF	DEN	I R P A	SAKD	F AW	AD <mark>A</mark> ۱	VFV	S GMH I Q	R EG Q		SR <mark>RAH</mark>	AAGI	(VAA	L GGP S V S	GAPEI	K Y P I	F D Y L	h∨gi	EIGDAT	DR	LIEIL	DRD	– L S	R P A/	a <mark>qv</mark> v	L D T K	ERL
co.	Methylobacterium organophilum	GV	< AFMP PQG L	L L I AA	YM P I	e A w e	CRF	DEN	I RR <mark>A</mark>	G P A D	F AW	AD <mark>A</mark> ۱	VFV	S GMH I Q	Q E P Q	THDI	RD <mark>R</mark> AF	AAGI	(VTV	L GGP SV:	GAPE	KYG	FD <mark>YL</mark>	HIGI	GDAT	DQ	VAR L	DGD	-VT	P P P C	o <mark>qv</mark> v	LETK	DRL
cteri	Rhodovolum sp. PH10	RVI	RALMP PQG <mark>L</mark>		S L P	< S <mark>W</mark> E	VRL	DEN	I R P A	TRD	F L W	AD <mark>A</mark> ۱	V L V	TGMHAQ		TEET	er <mark>ra</mark> f	ALGF	RTTV	L GGS SV	ACRE	FYP	S F <mark>D Y L</mark>	h∨gi	ELGDAT	DA		AKD	– P S	R P D F	R QV V	LETR	ERR
oba	Bradyrhizobium sp. BTAi1	GI	a f m p p q g <mark>l</mark>	L L <mark>I A</mark> A	Y L P	s s w q	VRF	DEN	RAA	QAED	E AW.	AE <mark>A</mark> ۱	VFV	S GMH I Q		MNDIC	oq <mark>raf</mark>	AFDI	. PVA	LGGPSV	ACPD	YYP	S FDYL	h∨gi	ELGDAT	DE		ARD	– P S	R P E C	2 <mark>97</mark> 7	L R T K	ERV
rote	Beijerinckia indica	GVI	KAFMP PQG <mark>L</mark>	lv <mark>ias</mark>	VMP	ANWD	VRF	DEN	/QA <mark>A</mark>	STED	F L W	AD <mark>۷</mark> ۱	V F V	S GMH I Q		IEDIF	RR <mark>RA</mark> F	AQGI	cvvv	LGGPSV	ACPH	YYP)	AFD <mark>YL</mark>	h∨gi	ELGDAT	ΕK		AKD	- I S	R P E G	2 <mark>97</mark> 7	LKTE	TRR
hap	Oligotropha carboxidovorans	G۷	< A F M P P Q G L	L L <mark>I A</mark> A	ALP	P S W S	VRF	DEN	I R S A	TDAD	E AW	AE <mark>A</mark> ۱	VFV	SGMHIQ	R K Q		CR <mark>RAH</mark>	EAEF	RVVA	L GGP SV	ACPD	YYPI	E F D <mark>Y L</mark>	нı gi	ELGDAT	EE	E F V R L	AD D	-VA	R P P F	R QV A	L TTH	IE R L
Alp	Methylocella silvestris	GV	(AFMPPQG <mark>L</mark>	l∨ <mark>iaa</mark>	ALP	2 H W G	VRF	DEN	/KR A	SAAD	F AW.	AD <mark>A</mark> ۱	VFV	S GMH I Q		MEDIO	CA <mark>RAH</mark>	EQGI	(P V A	L GGP S V S	AAPE	QYP/	A F D Y L	h∨gi	ELGDAT	EA	LIELI	AR D	-vs	R P P F	R <mark>Q I</mark> I	L K T K	DRR
	Afipia felis	GV	< AFMP PQG L	L L <mark>I</mark> AA	ALPI	e g w s	VRF	DEN	I RA <mark>A</mark>	TDED	F E W.	AE <mark>A</mark> ۱	V F V	SGMHIQ	RKQ		CR <mark>R</mark> AH	EHDI	РТА	L GGP SV	ACPD	YYPI	E F D <mark>Y L</mark>	HIGI	EMGDAT	DD	FARL	SAD	-РТ	R P K F	R QV M	LETH	IE R L
	Nitrobacter vulgaris	GV	QAFMPPQG <mark>L</mark>	L L <mark>I AA</mark>	Y L P	A D W N	VRF	DEN	RFA	TNED	F E W.	AE <mark>A</mark> ۱	VFV	SGMHIQ		MNDIC	er <mark>ra</mark> f	AFNI	. P V A	L GGP S V S	ACPD	YYP	T F D <mark>Y L</mark>	h∨gi	ELGDAT	DD		ARD	– P S	R P R S	s qv v	LKTA	.DR L
	Leptolyngbya boryanaCCAP 1462/2	v	AFMPPQGL	L I VAA	YLP	S E WD	VRF	DEN	I R P A	SRAD	YRW.	ADVI	VIV	SGMHIQ	RPQ		ALA	QAG	(TV	GGPSV	GCPE	YYP	FDIL	HLGI	ELGDAS	DR	41 E Y L	DRH	- H E	RPTO	R Q I R	FETV	DRL
	Cyanothece sp. PCC 7425	DV	RAFMPPQGI		YMP/	A SWE	VRLV	DEN	/TP A	TEAD	YRW	ADVI	V IT	S GMH I Q	RPQ			IRLG	(LTV	VGGPSV	GCPE	YYP	FDIL	HLGI	ELGDAT	DR	ALEY P	DLH	GST	RP P/	Q LR	FETU	ERL
	Nostoc punctiforme	NV	RAFMPPQGI		YLP	2 K W E	VRF	DEN	/KSA	TRAD	Y QW	ADA	viv	S GMH I Q	2K P Q		NEL AF	RAG	(T)	v <mark>ggpsv</mark> :	GCPE	YYPI	E F D I L	HLGI	ELGDAS	DR		DQN	– L E	R P Q F	R <mark>Q</mark> I R	FETK	ERL
eria	Cyanothece sp. PCC 7822	NV	R G F M P P Q G I	LVVAA	YLP	5 Q WE	VRF	DEN	I T L A	кккр	Y QW.	ADVI	viv	S GMH I Q	RPQ			AEG	(TV	GGPSV	GCPE	YYP	FDLL	H I GI	ELGDAT	DE	аткут	DRH	-HQ	RPA/	Q I R	FETR	ERL
acte	Prochlorothrix hollandica	RV	AFMPPQG I	L V V A S	YLPI	EEWE	VRF	DEN	(QP <mark>A</mark>	ктар	Y RW.	ADV	нт	SGMHIQ	R P Q		NEKAH	RQG	(I T L	VGGP SV	GCPE	YYP	FDLI	h∨gi	ELGDAT	DA	итенц	DHS	- I D	R P P S	5 q I R	FETK	DRL
anot	Cyanothece sp. PCC 7424	GV	AFMPPQG I	LV <mark>V</mark> AA	YLP	2QWE	VRF	DEN	A E A		YRW.	ADVI	viv	S GMH I Q	QRPQ		NDLA	REN	(LTV	v GGP S V :	ACPE	YYP	FDI	QIGI	ELGDGT	DQ	ALEY I	DRY	-CQ	R P E F	(Q C	FETK	DRL
Š	Scytonema hofmannii	RV	RAFMPPQGI	L V V A A	YLP	< QWE	VRF		/RS <mark>A</mark>	ĸĸsd	YRW	ADA	I V	S GMH I Q	REQ			TEG	(TV	VGGP SV:	GCPE	YYPI	FDIL	HLGI	EMGDAT	DQ		ртн	-TT	RP E P	QIR	FETK	ERL
	Gloeobacter violaceus	sv	RAFMPPQGI		YLP	4QWE	VRF	DEN	/IR P A	R S E D	Y RW.	ADAY	v i v	S GMH I Q	RPQ		NEL AH	RWG	(T A	LGGPSV	GCPE	YYP	FDLI	нı GI	ELGDAT	DR	ALEY I	DMH	- T E	R P A S	5 Q MR	FETA	ERL
	Gloeobacter kilauensis	v	RAFMPPQGI		YVP	A SWE	VRF	IDEN	I R P A	RMS D	Y QW.	ADAY	V IA	S GMH I Q	RRQ		5DL AH	RWG	(T A	VGGPSV	GCPE	YYP	FDLI	HIGI	ELGDAT	DQI	LIEY	DLH	- S E	R P A /	Q I R	LETK	ERL

Fig. S3. Alignment of the cobalamin-binding domain of HpnP amino acid sequences from selected

alphaproteobacteria and cyanobacteria. Highlighted are residues conserved in \geq 90% of either

99 cyanobacteria (green) or alphaproteobacteria (yellow). Also highlighted are residues universally

- 100 conserved in both clades (blue).
- 101

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102 **References**

- Altschul S. F., Gish W., Miller W., Myers E. W. and Lipman D. J. (1990) Basic local alignment search
 tool. *Journal of Molecular Biology* 215, 403–410.
- Crotti A. E. M., Lopes J. L. C. and Lopes N. P. (2005) Triple quadrupole tandem mass spectrometry of
 sesquiterpene lactones: a study of goyazensolide and its congeners. *Journal of Mass Spectrometry* 40, 1030–1034.
- Kool D. M., Talbot H. M., Rush D., Ettwig K. and Sinninghe Damsté J. S. (2014) Rare
 bacteriohopanepolyols as markers for an autotrophic, intra-aerobic methanotroph. *Geochimica et Cosmochimica Acta* 136, 114–125.
- Rohmer M., Bouvier-Nave P. and Ourisson G. (1984) Distribution of Hopanoid Triterpenes in
 Prokaryotes. *Microbiology* 130, 1137–1150.
- Sinninghe Damsté J. S., Rijpstra W. I. C., Dedysh S. N., Foesel B. U. and Villanueva L. (2017) Phenoand Genotyping of Hopanoid Production in Acidobacteria. *Frontiers in Microbiology* 8.
 Available at: http://journal.frontiersin.org/article/10.3389/fmicb.2017.00968/full [Accessed October 9, 2018].
- Talbot H. M., Watson D. F., Murrell J. C., Carter J. F. and Farrimond P. (2001) Analysis of intact
 bacteriohopanepolyols from methanotrophic bacteria by reversed-phase high-performance liquid
 chromatography–atmospheric pressure chemical ionisation mass spectrometry. *Journal of Chromatography A* 921, 175–185.
- Welander P. V., Coleman M. L., Sessions A. L., Summons R. E. and Newman D. K. (2010) Identification
 of a methylase required for 2-methylhopanoid production and implications for the interpretation
 of sedimentary hopanes. *Proceedings of the National Academy of Sciences* 107, 8537–8542.

- 124 Welander P. V. and Summons R. E. (2012) Discovery, taxonomic distribution, and phenotypic
- characterization of a gene required for 3-methylhopanoid production. *Proceedings of the National Academy of Sciences* 109, 12905–12910.