Cobaviruses – a new globally distributed phage group infecting *Rhodobacteraceae* in marine ecosystems

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Supplementary information

Experimental procedures

Cultivation media.

Liquid cultures of the bacterial host, *Lentibacter* sp. SH36 ([1]), were grown in artificial saltwater medium (1x ASW) (24.32 g/l NaCl, 10 g/l $MgCl_2x6H_2O$, 1.5 g/l $CaCl_2x2H_2O$, 0.66 g/l KCl, 4 g/l Na_2SO_4, 2.38 g/l HEPES, 0.6 g/l peptone, 0.3 g/l yeast extract, 84 mM KBr, 40 mM H_3BO_3, 15 mM $SrCl_2$, 40 mM NH_4Cl, 4 mM KH_2PO_4, 7 mM NaF, pH 7.5), which was autoclaved and completed before use with 1 ml/l of sterile filtered multi vitamin solution (after (Balch et al. 1979)), 0.25 ml/l of sterile filtered trace element solution A (1.5 g $FeCl_2x4H_2O$ in 10 ml 25 % HCl and 250 ml MilliQ water) and 0.1 ml/l of autoclaved trace element solution B (19 mg/l $CoCl_2x6H_2O$, 10 mg/l $MnCl_2x2H_2O$, 7 mg/l $ZnCl_2$, 3.6 mg/l $Na_2MoO_4x2H_2O$, 2.4 mg/l $NiCl_2x6H_2O$, 0.6 mg/l H_3BO_3, 0.2 mg/l $CuCl_2x2H_2O$).

The solid media used for plaque assays was Marine Broth. This media had the following recipe. 5.0 g/l peptone, 1.0 g/l yeast extract, 0.1 g/l $C_6H_8FeO_7$, 12.6 g/l $MgCl_2x6H_2O$, 3.24 g/l Na_2SO_4, 19.45 g/l NaCl, 2.38 g/l $CaCl_2x2H_2O$, 0.55 g/l KCl, 0.16 g/l $NaHCO_3$, 0.01 g/l $Na_2HPO_4x2H_2O$, 0.008 g/l KBr, 0.034 g/l $SrCl_2x6H_2O$, 0.022 g/l H_3BO_3, 0.007 g/l $Na_2SiO_3x3H_2O$, 0.0024 g/l NaF, 0.0016 g/l NH_4NO_3.

Purification of ICBM1 and ICBM2 phages.

Bacterial cells and their debris from the phage enrichments were removed by a centrifugation step (15 min, 4000 x g, 20 °C), followed by 0.2 µm filtration (0.2 µm, Rotilabo-syringe filters, Carl Roth) of the supernatant. The ICBM1 and ICBM2 phages were isolated from the enriched phage fractions by obtaining single phage plaques.

To obtain single plaques, serial dilutions (10^0, 10^{-1}, etc.) were prepared from the phage fractions by mixing with ASW base (ASW media without yeast extract, peptone and vitamins). 100 µl of phage dilution were mixed with 280 µl of exponentially growing host culture (OD = 0.2-0.3) and incubated for 15 min on ice. Afterwards, the mixture was transferred to 3 ml MB-soft agar (0.6 % low melting point Biozym Plaque GeneticPure agarose, Biozym, kept warm at 37°C), mixed by brief vortexing and poured onto the bottom MB agar layer (1.8 % agar). After drying of the top layer, the plates were incubated for three days at 20 °C. For isolation, when phage plaques were observed as clearing zones within the grown bacterial lawn, they were picked with sterile Pasteur pipettes and incubated overnight in 500 µl ASW base at 4 °C. After subsequent centrifugation (10 min, 10000 x g, 4 °C), the supernatant was used for a next round of plaque assays. This procedure of plaque assay, picking of plaques and re-plating was repeated three times to ensure purity of the newly isolated phages.

The ICBM1 and ICBM2 phages were stored either as phage lysate at +4°C or as glycerol stock of free phages or infected cells at -80°C (for details, see below).

Spot assays.

To estimate phage numbers, serial dilutions (10^0, 10^{-1}, etc.) were prepared from the phage fractions by mixing with ASW base (ASW media without yeast extract, peptone and vitamins). 280 µl of exponentially growing host culture (OD = 0.2-0.3) were mixed with 3 ml MB-soft agar (kept warm at 37°C) and poured onto the bottom MB agar layer. After drying of the top layer, 10 µl of each phage fraction dilution were pipetted on top as droplets. The plates were incubated at 20 °C.

Preparation of phage ICBM1 and ICBM2 stocks.

In two Erlenmeyer flasks, 20 ml 1xASW medium was inoculated with an exponentially growing *Lentibacter* sp. SH36 culture (final OD = 0.006). One culture was infected with 300 µl of the phage ICBM1 supernatant, the other was not infected and regarded as control. Both cultures were incubated at 20 °C and 100 rpm overnight, until bacterial lysis in the infected culture was indicated by low OD_{600}

(compared with the control culture) and disrupted cell particles. The phage fraction was obtained by removing cells and debris by centrifugation (15 min, 4000 x g, 20 °C), followed by 0.22 µm filtration of the supernatant. The phage fraction was stored at +4°C. For long term storage, two types of glycerol stocks were prepared: i) stock of free phage particles (1 part phage fraction and 1 part MB media with 50% glycerol) and ii) stock of infected host cells (1 part infected cells - 375 µl phage fraction added to 375 µl host culture, 15 min on ice for absorption - and 1 part MB media with 50% glycerol).

TEM.

Phages were concentrated from the ICBM1 or ICBM2 phage lysate by polyethylene glycol (PEG) precipitation. 150 ml phage fraction resulting from two subsequent infections (see above) were incubated for 2 h at 4 °C with PEG (final concentration 10%) and NaCl (final concentration 0.6 mM). After centrifugation for 2 h at 7197 x g and 4 °C, the supernatant was discarded and the pellet resuspended in 500 µl SM buffer (100 mM NaCl, 8 mM MgSO4, 50 mM Tris-HCl pH 7.4) in total. For resuspension of the phages, 30 min incubation at 4 °C followed. PEG was removed by mixing with an equal volume of 100 % chloroform, shaking for 5 min and incubation on ice for 5 min. After centrifugation (10 min, 3000 x g, 4 °C), the upper layer was collected.

Further concentration and purification of the phages was done by cesium chloride gradient ultracentrifugation. In UltraClear™ centrifuge tubes, a density gradient was set up from cesium chloride solutions with different densities (from bottom up): 1.5 ml of 1.65 g/ml, 2 ml of 1.5 g/ml, 2 ml of 1.4 g/ml, 1 ml of 1.2 g/ml. The PEG concentrated phage fraction was transferred on top. Ultracentrifugation was run for 4 h at 20 °C and 25000 rpm (Beckman, SW 41 Ti). Afterwards, the visible band corresponding to the phages was collected with syringe and needle through the side wall of the ultracentrifuge tube (~500 µl). Removal of cesium chloride was done by dialysis with Slide-A-Lyzer® G2 Dialysis Cassettes 10K MWCO (ThermoScientific) against ASW base for 21 h with buffer exchange after 3 h and 18 h.

30 µl of phage ICBM1 or ICBM2 concentrate were pipetted on top of a carbon coated grid (Formvar 162, 200 mesh) and phages were allowed to absorb for 3 min, followed by staining with 30 µl uranylacetate (2 %) or ammonium molybdate (2 %) for 45 sec and gentle removal of the liquid with filter paper. After air drying for 15 min, the grids were visualized with the transmission electron microscope Zeiss EM902A. Images were documented with the Proscan High Speed SSCCD camera and analyzed using the software ImageSP viewer (Version 1.2.5.16).

DNA extraction from the ICBM1 phage.

Phages were concentrated from the phage lysates by precipitation with polyethylene glycol. For this purpose, 4 x 25 ml phage lysate prepared as above (with one exception: to avoid phage loss, cells and debris were removed only by centrifugation, and not by filtration) were mixed with 50% PEG (final concentration 10 %) and 5M sodium chloride (final concentration 0.6 M) and incubated for 2 h at 4 °C. After centrifugation (2 h, 7197 x g, 10 °C) the phage pellets were resuspended in 500 µl SM buffer (100 mM NaCl, 8 mM MgSO4, 50 mM Tris-HCl pH 7.4) each. Extracellular DNA was removed by incubating the phage concentrates with 0.04 units/µl of Turbo DNase (Invitrogen, Ambion) for 30 min at 37°C, followed by enzyme inactivation by incubating for 10 min at 75 °C with 15 mM EDTA. Further, the phage DNA was extracted using the ChargeSwitch gDNA Mini Bacteria Kit (ThermoFisher Scientific), according to the instructions manual, including the with the RNase digestion, but with the exception of no lysosyme in the first step. The DNA was finally eluted in 1 ml elution buffer. The concentration and quality of the obtained DNA was checked fluorometrically with Qubit 2.0 and the Qubit® dsDNA

HS Assay, spectrophotometrically with Nanodrop 2000 spectrophotometer and visually by regular gel electrophoresis (0.7 % agarose gel, 50 V, SYBR Gold staining).

DNA extraction of the intracellular phage fraction from the phage enrichments.

To extract the intracellular phage fraction from the phage enrichment, when lysis was observed, the cells from the enrichments and the positive control were retrieved by centrifugation (15 min, 4000 x g, 20 °C). The cell pellets were embedded in agarose plugs by mixing with SeaKem Gold Agarose for PFGE, Lonza Rockland Inc. (final concentration 0.8 %), distributing the mixture into 100 µl molds and allowing it to solidify for 30 min at 4 °C. Plugs were collected in a 50 ml Falcon tube and incubated overnight at 50 °C in 2 ml ESP buffer (1 % N-laurylsarcosine, 1 mg/ml proteinase K, 0.5 M EDTA pH 9.0). Afterwards, the ESP was discarded, the plugs were washed three times with TE buffer (10 mM Tris-HCl pH 8.0, 2 mM EDTA pH 9.0) and stored in TE buffer at 4 °C until further use. The DNA from the agarose plugs was separated during agarose gel electrophoresis (1 % SeaPlaque GTG Agarose, Lonza Rockland Inc., TAE buffer - 40 mM Tris-acetate, 1 mM EDTA, pH 8.3, migration 2 h at 60 V). Afterwards, the gel was cut into two halves. One half was stained with Ethidium bromide bath (1 µg/ml, 30 min) and documented with BioDocAnalyze system (Biometra). The distance from the loading pocket to the phage DNA band was measured and used to localize the phage DNA band in the unstained half and to cut it out from the gel. The agarose pieces were stored overnight in TE buffer (10 mM Tris-HCl pH 8.0, 5 mM EDTA pH 9.0), followed by agarose digestion with 1 unit of β-agarase (New England Biolabs Inc.), according to the manufacturer's protocol. Undigested agarose was removed by centrifugation for 10 min at 20,000 x g. The obtained supernatants were concentrated to 100 µl each using 100 kDa Amicon Ultra centrifugal filters (0.5 ml volume, Merck Millipore) and then stored at -20 °C. Afterwards, an additional purification step was performed using the ChargeSwitch gDNA Mini Bacteria Kit (ThermoFisher Scientific) for DNA extraction. This was done according to the kit manual (including the RNase digestion, but without lysozyme) and the DNA was finally eluted in 100 µl elution buffer. Concentration and quality of the obtained DNA were checked with Qubit 2.0 fluorometer, Nanodrop 2000 spectrophotometer and by regular gel electrophoresis (0.7 % agarose gel, 50 V, Ethidium bromide staining).

Illumina genome sequencing.

The extracted DNA from the ICBM1 and ICBM2 phages and the phage enrichments was used to generate Illumina NexteraXT shotgun paired-end sequencing libraries, which were sequenced with a MiSeq instrument and the MiSeq reagent kit version 3, as recommended by the manufacturer (Illumina, San Diego, CA, USA). For quality-filtering, Trimmomatic version 0.35 (Bolger et al. 2014) or Bbduk from the BBTools package (BBTools (https://jgi.doe.gov/data-and-tools/bbtools/) were used. The assembly was performed with the SPAdes genome assembler software version 3.9.0 (Bankevich et al. 2012) and the read coverage of the whole assembly determined with QualiMap version 2.1 (García-Alcalde et al. 2012). In addition, the read mapping of enrichments reads on individual phage genomes was done BBMap from BBTools package.

PacBio library preparation, sequencing and assembly.

SMRTbell template library was prepared according to the instructions from Pacific Biosciences, Menlo Park, CA, USA, following the Procedure & Checklist Greater than 10 kb Template Preparation and Sequencing. Briefly, for preparation of 10kb libraries ~4µg of each phage DNA was sheared using a Covaris S220, Woburn, MA, USA according to the manufacturer´s instructions. DNA was end-repaired and ligated overnight to barcoded SMRTbell adapters applying components from the DNA/Polymerase

Binding Kit P6 from Pacific Biosciences (Menlo Park, CA, USA). Reactions were carried out according to the instructions of the manufacturer. One part VB-1 SMRTbell template was combined with 2.5 parts Ex53-3 SMRTbell template. BluePippin Size-Selection to greater than 4 kb was performed according to the manufacturer´s instructions (Sage Science, Beverly, MA, USA). Conditions for annealing of sequencing primers and binding of polymerase to purified SMRTbell template were assessed with the Calculator in RS Remote, PacificBiosciences, Menlo Park, CA, USA. SMRT sequencing was carried out on the PacBio *RSII* (PacificBiosciences, Menlo Park, CA, USA) taking one 240-minutes movie for one SMRT cell. Long read genome assemblies of all three phages have been performed using the HGAP4 Whitelisting protocol (https://github.com/PacificBiosciences/Bioinformatics-Training/wiki/HGAP-Whitelisting-Tutorial) within SMRTPipe 2.3.0 applying a genome size of 100kb and a minimum subread length of 1kb after demultiplexing using the RS_Subreads.1 protocol contained within SMRT Portal 2.3.0.

Results

Retrieval of similar phage genomes from public sequence datasets.

To find cultured and environmental relatives of the three phages, the following datasets were queried for related sequences: i) the Tara Ocean Viromes (Brum et al. 2015), ii) the Earth Virome (Paez-Espino et al. 2016), iii) the Global Ocean Virome (GOV, (Roux et al. 2016)), iv) the IMG/VR (Paez-Espino et al. 2017) and v) the Environmental Viral Genomes (Nishimura et al. 2017). Six highly similar genomes belonged to cultivated phages: the P12053L phage (38889 bp) infecting *Celeribacter marinus* IMCC12053^{T}, the SIO1 phage (39 898 bp) infecting *Roseobacter* sp. SIO67 and four other SIO1 related strains, infecting *Roseobacter* sp. SIO67 and *Roseobacter* sp. GAI-101 (Angly *et al.* 2009). The last four phages had incomplete genomes, with several regions of uncertainty (long N stretches). Therefore, they were included in the phylogenetic analysis as draft genomes, but excluded from further genomic analysis. Several other genomes represented environmental fragments of different lengths. Only complete or close to complete genomes (>35 kbps, no long N stretches) were considered for further phylogenetic and genomic analyses. One of these circularized due to terminal redundancies, indicating genome completeness - EnvX (40752 bp), and five of them were incomplete, but close in size to the complete genomes and contiguous - Env9 (41607 bp), EnvY (36003 bp), EnvZ (35824 bp), Env8 (38447 bp) and Env14 (35006 bp). EnvX, EnvY, EnvZ, Env8 and Env9 were retrieved from IMG/VR /Earth Virome datasets. Env14 was retrieved from the GOV dataset (see Table 2).

Genome termini and genome rearrangements..

ICBM1, ICBM2 and ICBM3. We sequenced the three phages by Illumina and PacBio, as isolates or from the enrichments (Table S2). The three phage genomes were assembled as circular contigs of ~ 40kbps. Sequencing coverage data was used to determine the ends of the genomes. With Illumina sequencing, a sharp drop in coverage was noticed for ICBM1 and ICBM2 genomes, which could indicate linear, non-circularly permuted genomes, with cohesive ends (Merrill et al. 2016), (Figure S3). On the other hand, for both genomes, we noticed a region of low coverage and high %GC (see Figure S3). The low coverage could be due to the library preparation with the NexteraXT kit, which is known to have low performance at the genome ends (Illumina 2015). Furthermore, the high %GC could contribute to the drop in coverage (Aird et al. 2011). To elucidate the genome ends, we turned to PacBio single molecule real time (SMRT) sequencing, because native DNA is used and thus, no PCR bias is observed. Furthermore, much longer read lengths can be retrieved, which facilitates assembly, especially important for mixed samples, for example the phage enrichments. Thus, the artificial redundancies produced by the assembler at the end of the three phage contigs were larger, having a size of ~5kb, which is equal to the mean subread length achieved with our PacBio sequencing approach (Figure S4). In all three phage genomes, short DTRs of about 160 bps were easily recognized as spikes in coverage (Figure S4 A). To delineate the final genome structure of the three phages, artificial redundancies were removed and the phage genome was adjusted to their direct terminal repeats (Figure S4 C). The exact genome start and stop positions were derived from long read mappings by a detailed inspection of the respective regions in Integrative Genome Viewer (Robinson et al. 2011) (Figure S5). The sharp drop in coverage in the Illumina assemblies corresponded to a GC rich region of the DTRs (see Figure S3), and thus, explained the apparent discrepancy between the Illumina and PacBio read coverage data.

We used the DTRs from ICBM1 to define the genome termini and rearrange the gene order accordingly, not only for the SIO1 phage, but also for the P12053L and environmental cobaviruses.

SIO1. A search with the ICBM1 DTR in the SIO1 genome revealed the presence of a similar region (80% nucleotide identity) at position 8716-8891. Based on this approach, base 8716 from the original

SIO1 genome became base 1 in the reordered genome, with the left side being concatenated at the end of the right side, and the ICBM1 phage DTR homologous region being added also at the right end, as depicted in Fig. S5. Similar regions were found in all four SIO1-related phages isolated by (Angly et al. 2009), having 90% to 99% nucleotide identity with the SIO1 DTR.

P12053L. A search with the ICBM1 DTR in the P12053L genome found a similar region (~94% identity) at position 244-415. Therefore, the genome was rearranged in a similar way to SIO1, with base 244 becoming base 1. No gene rearrangement was necessary in this case. The DTR sequence was determined by homology to ICBM1 DTR and added at the right end of the genome also.

Environmental cobaviruses. For two of the environmental genomes (EnvX and Env9) we determined the genome start by finding regions with high identity with the left region of the ICBM2 DTR (>70% over 49 nt, see Figure S9). However, the complete sequence of the DTRs could not be established, because of the low similarity over the remaining alignment (~50% identity). Env9 was not circular, but because we established the start at position 3404, we plotted the position 1-3403 at the end of the genome in Figure 3. The remaining environmental genomes showed no regions of similarity with the ICBM1 or ICBM2 phage DTRs, presumably due to their incompleteness. The phylogenetic positioning both in the GBPD and terminate trees strongly support the presence of DTRs at as genome termini for all the environmental genome.

234 **Table S1: List of *Rhodobacteraceae* strains used for the host range assay.**

Name	Strain designation	Strain	Infected (- no, + yes)
Antarctobacter heliothermus	EL-219	DSM 11445	-
Celeribacter baekdonensis	L-6	DSM 27375	-
Celeribacter indicus	P73	DSM 27257	-
Celeribacter marinus	IMCC12053	DSM 100036	-
Celeribacter neptunius	H 14	DSM 26471	-
Citreicella aestuarii	AD8	DSM 22011	-
Citreicella marina	CK-I3-6	DSM 26424	-
Dinoroseobacter shibae	5 Plasmids	DSM 16493	-
Huaishuia halophila	ZXM137	DSM 26270	-
Hwanghaeicola aestuarii	Y26	DSM 22009	-
Jannaschia donghaensis	DSW-17	DSM 102233	-
Jannaschia helgolandensis	Hel10	DSM 14858	-
Jannaschia pohangensis	H1-M8	DSM 19073	-
Jannaschia rubra	4SM3	DSM 16279	-
Leisingera aquimarina	R-26159	DSM 24565	-
Leisingera caerulea	13	DSM 24564	-
Leisingera daeponensis	TF-218	DSM 23529	-
Leisingera methylohalidivorans	MB2	DSM 14336	-
Lentibacter sp.	SH36		
Litoreibacter albidus	KMM 3851	DSM 26922	-
Litoreibacter arenae	GA2-M15	DSM 19593	-
Litoreibacter janthinus	KMM 3842	DSM 26921	-
Litorimicrobium taeanense	G4	DSM 22007	-
Loktanella cinnabarina	LL-001	DSM 29954	-
Loktanella fryxellensis	R-7670	DSM 16213	-
Loktanella hongkongensis	UST950701-009P	DSM 17492	-
Loktanella koreensis	GA2-M3	DSM 17925	-
Loktanella pyoseonensis	JJM85	DSM 21424	-
Loktanella salsilacus	R-8904	DSM 16199	-
Loktanella tamlensis	SSW-35	DSM 26879	-
Loktanella vestfoldensis	R-9477	DSM 16212	-
Maribius pelagius	B5-6	DSM 26893	-
Maribius salinus	CL-SP27	DSM 26892	-
Marinovum algicola	FF3	DSM 10251	-
Marinovum algicola	DG898	DSM 27768	-
Maritimibacter alkaliphilus	HTCC2654	DSM 100037	-
Nautella italica	R11	DSM 26436	-
Oceanibulbus indolifex	HEL-45	DSM 14862	-
Oceanicola batsensis	HTCC2597	DSM 15984	-
Oceanicola granulosus	HTCC2516	DSM 15982	-
Oceanicola nanhaiensis	SS011B1-20	DSM 18065	-
Octadecabacter temperatus	SB1	DSM 26878	-
Palleronia marisminoris	B33	DSM 26347	-
Pelagibaca bermudensis	HTCC2601	DSM 26914	-
Phaeobacter gallaeciensis	BS 107	DSM 26640	-
Phaeobacter inhibens		DSM 17395	-

Phaeobacter inhibens	T5	DSM 16374	-
Phaeobacter inhibens	2.10	DSM 24588	-
Ponticoccus litoralis	CL-GR66	DSM 18986	-
Pseudophaeobacter arcticus	20188	DSM 23566	-
Pseudoruegeria lutimaris	HD-43	DSM 25294	-
Roseibacterium elongatum	Och 323	DSM 16469	-
Roseivivax isoporae	sw2	DSM 22223	-
Roseivivax roseus	BH87090	DSM 23042	-
Roseobacter denitrificans	Och 114	DSM 7001	-
Roseobacter litoralis	Och 149	DSM 6996	-
Roseovarius crassostreae	CV919-312, CVSP	DSM 16950	-
Roseovarius halocynthiae	MA1-10	DSM 27840	-
Roseovarius indicus	B108	DSM 26383	-
Roseovarius lutimaris	112	DSM 28463	-
Roseovarius marinus	HDW-9	DSM 25228	-
Roseovarius mucosus	DFL-24	DSM 17069	-
Roseovarius nubinhibens	ISM	DSM 15170	-
Ruegeria atlantica	1480	DSM 5823	-
Ruegeria conchae	TW15	DSM 29317	-
Ruegeria marina	ZH17	DSM 24837	-
Ruegeria pomeroyi	DSS-3	DSM 15171	-
Sagittula stellata	EE-37	DSM 11524	-
Salinihabitans flavidus	ISL-46	DSM 27842	-
Salipiger mucosus	A3	DSM 16094	-
Sedimentitalea nanhaiensis	NH52F	DSM 24252	-
Sediminimonas qiaohouensis	YIM B024	DSM 21189	-
Shimia haliotis	WM35	DSM 28453	-
Shimia marina	CL-TA03	DSM 26895	-
Sulfitobacter delicatus	KMM 3584	DSM 16477	-
Sulfitobacter dubius	KMM 3554	DSM 16472	-
Sulfitobacter litoralis	Iso 3	DSM 17584	-
Sulfitobacter marinus	SW-265	DSM 23422	-
Sulfitobacter mediterraneus	CH-B427	DSM 12244	-
Sulfitobacter noctilucae	NB-68	DSM 100978	-
Sulfitobacter noctilucicola	NB-77	DSM 101015	-
Sulfitobacter pseudonitzschiae	H3	DSM 26824	-
Sulfitobacter sp.	EE-36	DSM 11700	-
Thalassobius aestuarii	JC2049	DSM 15283	-
Thalassobius maritimus	GSW-M6	DSM 28223	-
Thalassococcus halodurans	UST050418-052	DSM 26915	-
Thioclava dalianensis	DLFJ1-1	DSM 29618	-
Thioclava pacifica	TL 2	DSM 10166	-
Tranquillimonas alkanivorans	A34	DSM 19547	-
Tropicibacter multivorans	MD5	DSM 26470	-
Tropicibacter naphthalenivorans	C02	DSM 19561	-
Tropicimonas isoalkanivorans	B51	DSM 19548	-
Wenxinia marina	HY34	DSM 24838	-
Yangia pacifica	DX5-10	DSM 26894	-

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Table S2: Sequencing and assembling phage genomes from isolates and enrichments

	Phage isolates – sequenced by		Enrichments – sequenced by	
	Illumina	**PacBio**	**Illumina (S1 and S2)**	**PacBio (S2 only)**
ICBM1	yes	yes	not assembled	not assembled
ICBM2	yes	no	assembled in S2	assembled in S2
ICBM3	n.a.	n.a.	assembled in S1, S2	assembled in S2

Table S3: Cobaviral genomes - % GC and length (bases).

Name	%GC	Sequence Length (bases)
vB_LenP_ICBM1	47.00%	40163
vB_LenP_ICBM2	47.80%	40907
vB_LenP_ICBM3	47.30%	40498
SIO1	46.20%	40072
P12053L	46.10%	39061
EnvX	40.20%	40752
EnvY	40.10%	36003
EnvZ	44.80%	35824
Env8	39.80%	38447
Env9	40.30%	41607
Env14	40.30%	35066

Table S4: Bi-directional rho-independent transcriptional terminators in the genomes of the Cobavirus group (blue - stems, red - loops).

phage	Terminator name	Position	sequence	strand	delta G
ICBM1	t1	15927-15979	CAAATAAGTAAAGCCCCCAAGGAGAAATCCAA GGGGGCTaTTTCTTTGTGTAT	-	-13.84
ICBM1	t2	15930-15981	CACAAAGAAATAGCCCCCTTGGATTTCTCCTTGG GGGCTTTACTTATTTGGA	+	-13.64
ICBM3	t1	15916-15968	CAAATAAGTAAAGCCCCCAAGGAGAAATCCAA GGGGGCTaTTTCTTTATGTAT	-	-13.84
ICBM3	t2	15919-15970	CATAAAGAAATAGCCCCCTTGGATTTCTCCTTGG GGGCTTTACTTATTTGGA	+	-13.64
ICBM2	t1	14733-14782	AACACAAAGAAGCCCCCAAGGAGAAATCCAAG GGGGCTTTTGCTTGTCTA	-	-12.64
ICBM2	t2	14735-14784	GACAAGCAAAAGCCCCCTTGGATTTCTCCTTGG GGGCTTCTTTGTGTTTA	+	-13.14
P12053L	t1	17261-17316	TAAACACAAAGAAGCCCCCAAGGATTTTACTCC AAGGGGGCTTTTGCTTGTTCATC	-	-14.09
P12053L	t2	17265-17316	AACAAGCAAAAGCCCCCTTGGAGTAAAATCCTT GGGGGCTTCTTTGTGTTTA	+	-12.99
SIO1	t1	15149-15204	TAAACACAAAGAAGCCCCCAAGGATTAATCTCC AAGGGGGCTTTTGTTTGTCTATA	-	-14.09

SIO1	t2	15153-15204	GACAAACAAAAGCCCCCTTGGAGATTAATCCTTGGGGGCTTCTTTGTGTTTA	+	-12.99
EnvX	t1	12131-12185	GAAATAAAGAAGAAGCCCCAAGGAGAAATCCTGAGGGGCTTTTTTATTACTCTTG	-	12.06
EnvX	t2	12134-12187	GAGTAATAAAAAAGCCCCTCAGGATTTCTCCTTGGGGCTTcTTCTTTATTTCTT	+	-11.26
EnvY	t1	12048-12102	GAAATAAAGAAGAAGCCCCAAGGAGAAATCCTGAGGGGCTTTTTTATTACTCTTG	-	-12.06
EnvY	t2	12051-12104	GAGTAATAAAAAAGCCCCTCAGGATTTCTCCTTGGGGCTTcTTCTTTATTTCTT	+	-11.26
Env8	t1	11722-11764	AGAAAAAGTAAGGGAGCCTAAGTAGCTCCCcTTTTTTATACCT	-	-10.60
Env8	t2	11724-11765	GTATAAAAAAGGGGAGCTACTTAGGCTCCCTTACTTTTTCTT	+	-12.70
Env9	t1	18115-18164	AAATAAAATAAACCCCCTTGGATTTCTCCTTGGGGGTTTTTTCTTACTTG	-	-10.44
Env9	t2	18115-18169	CAAGTAAGAAAAAACCCCCAAGGAGAAATCCAAGGGGGTTTaTTTTATTTCTTTT	+	-12.34
Env14	t1	12255-12297	TAAAAGAAGAAGGGAGCCTAAGTAGCTCCCcTTTTTTTATGC	-	-10.60
Env14	t2	12257-12298	ATAAAAAAAAGGGGAGCTACTTAGGCTCCCTTCTTCTTTTAA	+	-12.70

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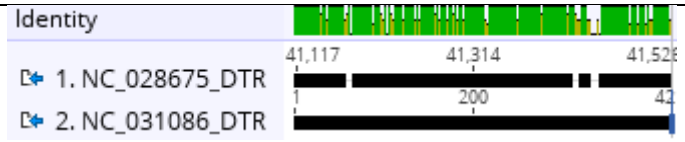
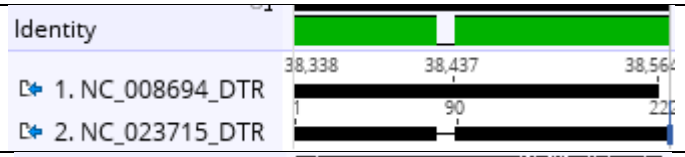
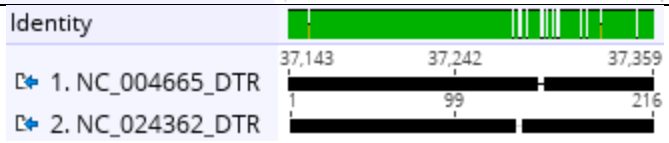
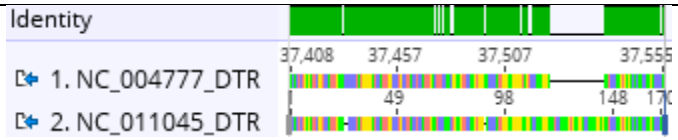
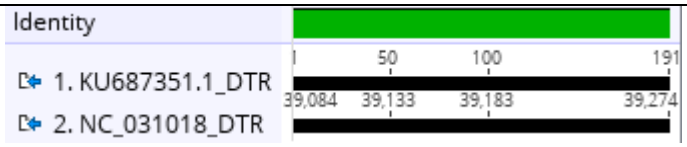
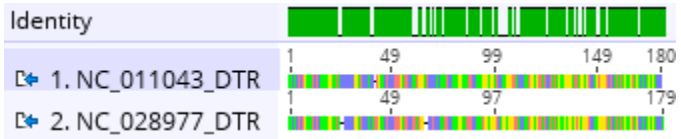
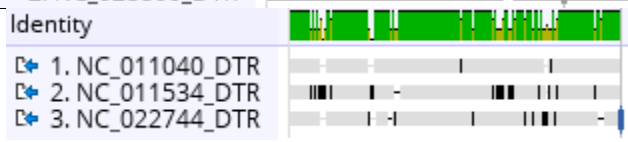
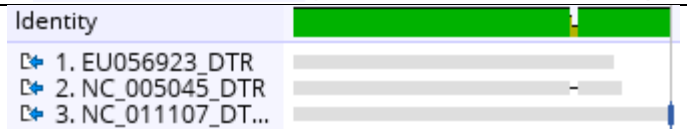
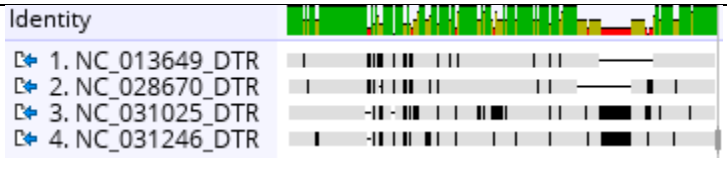
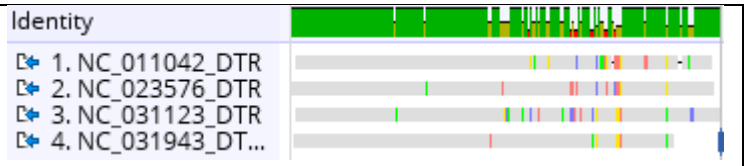
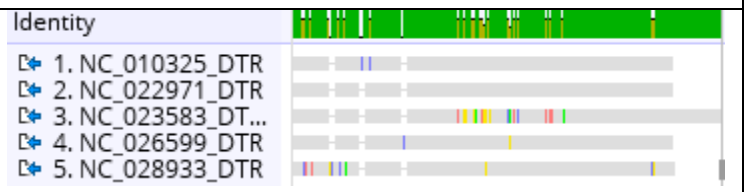
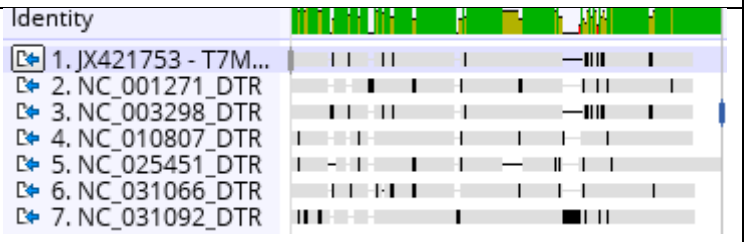
Table S5: DTRs from related phages.

Group	phages	DTRs - length	DTRs - nucleotide identity (excluding gaps)	DTRs - alignment
1	Acinetobacter phage phiAB1	410	87%	
	Acinetobacter phage phiAB6	421		
2	Yersinia phage Berlin	227	95%	
	Yersinia phage Yep-phi	222		
3	Pseudomonad phage gh-1	217	92%	
	Pseudomonas phage phiPSA2	216		
4	Yersinia phage phiA1122	148	80%	
	Enterobacteria phage 13a	170		
5	Citrobacter phage SH5	191	100%	
	Citrobacter phage SH4	191		
6	Klebsiella phage K11	180	83%	
	Klebsiella phage vB_KpnP_KpV289	179		
7	Enterobacteria phage K30	393	80%	
	Klebsiella phage K5	392		
8	Enterobacteria phage BA14	194	83% - 93%	
	Kluyvera phage Kvp1	194		
	Erwinia phage FE44	193		
9	Pseudomonas phage PT5	413	98% - 100%	
	Pseudomonas phage phiKMV	414		
	Pseudomonas phage PT2	488		
10	Klebsiella phage KP34	216	71% - 87%	
	Klebsiella phage vB_KpnP_KpV41	214		
	Klebsiella phage vB_KpnP_KpV475	243		
	Klebsiella phage vB_KpnP_KpV71	246		

11	Enterobacteria phage EcoDS1	178	84% - 93%	
	Citrobacter phage CR44b	183		
	Citrobacter phage SH3	184		
	Escherichia phage vB_EcoP_GA2A	165		
12	Pseudomonas phage LUZ24	184	88% - 99%	
	Pseudomonas phage phiIBB-PAA2	183		
	Pseudomonas phage TL	207		
	Pseudomonas phage vB_PaeP_C2-10_Ab22	184		
	Pseudomonas phage PhiCHU	185		
13	Enterobacteria phage T7M	230	83% - 100%	
	Yersinia phage phiYeO3-12	232		
	Enterobacteria phage T3	231		
	Salmonella phage phiSG-JL2	230		
	Yersinia phage vB_YenP_AP5	235		
	Citrobacter phage SH1	231		
	Citrobacter phage SH2	243		

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249 **Table S6: Prophage predictions using Phaster for the contigs containing the spanin gene**

Organism	Contig accession	Predicted prophage position	Prophage score	Spanin position	Spanin accession	Spanin associated to prophage
Thalassobius gelatinovorus strain DSM 5887	NZ_FOFW01000006	189621-213252	intact	208342..208716	WP_058264408.1	yes
Salinihabitans flavidus strain DSM 27842	FODS01000069	none, too short contig		1323..1676	SEP27667	unknown
Ruegeria mobilis strain DSM 23403	NZ_FNNK01000005	157938-194421	intact	178515..178898	WP_065332003	yes
Ruegeria mobilis strain M41-2.2	NZ_LNWW01000004	700765-722736	incomplete	721841..722224	WP_065329472	yes
Silicibacter sp. TM1040	NC_008044.1	1376648-1397748	intact	1396955..1397338	WP_011538643	yes
Rhodovulum sp. MB263	NZ_CP020384	862737-879872	incomplete	865553..865897	WP_080615430	yes
Rhodovulum sulfidophilum strain AB26	MSYQ01000001	many, not associated with the spanin		1904888..1905244	OLS44559.1	no
Phaeobacter sp. P97	NZ_CP016364	1925407-1946347	incomplete	1945236..1945619	WP_072504847	yes
Phaeobacter inhibens strain S4Sm	NZ_LOHU01000025	4300-27092	questionable	5028..5411	WP_061047696	yes
Phaeobacter sp. S26	NZ_JSWK01000008	125831-162508	questionable	140888..141271	WP_040172280	yes
Phaeobacter inhibens DSM 16374	NZ_KI421498	1832819-1867875	intact	1854039..1854422	WP_027247877	yes
Phaeobacter inhibens DSM 17395	NC_018290.1	1905561-1926725	intact	1925596..1925973	WP_014880219	yes
Leisingera sp. ANG-M7	NZ_JWLI01000008.1	60402-97630	intact	77885..78262	WP_052272404	yes

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Table S7: Environmental distribution of *Celeribacter marinus* IMCC 12053, based on the 16S rRNA blast hits in the NR Blast databases from NCBI, with minimum 99.0% identity.

Blast hit Accession	Isolation source	Country	coordinates	% nucleotide Identity	Alignment length	evalue
AJ391195.1	Adriatic Sea			100	1338	0
AJ391196.1	Adriatic Sea			100	1360	0
CP012023.1	coastal surface seawater of the Yellow Sea	South Korea		100	1471	0
AM990783.1	sea water North Western Mediterranean Sea	France	42.29 N 3.08 E	99.858	1407	0
AY697903.1	seawater	Antarctica		100	1410	0
NR_133717.1	seawater, Yellow Sea	South Korea		99.853	1359	0
HM140667.1	toxigenic diatom Pseudo-nitzschia, Puget Sound	WA (Main Basin)		99.29	1409	0
GU061042.1	Yellow Sea intertidal beach	Korea		99.283	1394	0
GU061048.1	Yellow Sea intertidal beach	Korea		99.211	1394	0
HM140672.1	Puget Sound	WA (Main Basin)		99.219	1409	0
HM140674.1	Puget Sound	WA (Main Basin)		99.148	1409	0

Table S8: Environmental distribution of *Lentibacter* sp. SH36, based on the 16S rRNA blast hits in the NR Blast databases from NCBI, with minimum 99.0% identity.

Blast hit Accession	Isolation source	Country	coordinates	% nucleotide Identity	Alignment length	evalue
AJ391182.1	Adriatic Sea			100	1429	0
JQ269272.1	estuary zone of Jiulong River	China		99.508	1422	0
AF305498.1	German bight			99.502	1405	0
AY701455.1	Gymnodinium catenatum from Huon Estuary,	Tasmania (Australia)		99.71	1381	0
DQ234098.2	mangrove, Danshui river estuary	Northern Taiwan		99.862	1452	0
DQ234152.2	mangrove, Danshui river estuary	Northern Taiwan		99.931	1452	0

DQ234180.2	mangrove, Danshui river estuary	Northern Taiwan		99.931	1452	0
DQ234196.2	mangrove, Danshui river estuary	Northern Taiwan		99.931	1452	0
DQ234202.2	mangrove, Danshui river estuary	Northern Taiwan		99.862	1452	0
DQ234210.2	mangrove, Danshui river estuary	Northern Taiwan		99.931	1452	0
DQ234244.2	mangrove, Danshui river estuary	Northern Taiwan		99.862	1452	0
AY145564.1	marine section of the Weser estuary	Germany		99.778	1350	0
EU799040.1	Newport Harbour, RI	USA	41.486 N 71.351 W	99.86	1428	0
EU799044.1	Newport Harbour, RI	USA	41.486 N 71.351 W	99.93	1428	0
EU799171.1	Newport Harbour, RI	USA	41.486 N 71.351 W	99.72	1428	0
EU799468.1	Newport Harbour, RI	USA	41.486 N 71.351 W	100	1403	0
EU799546.1	Newport Harbour, RI	USA	41.486 N 71.351 W	99.79	1428	0
EU799658.1	Newport Harbour, RI	USA	41.486 N 71.351 W	99.86	1428	0
EU800046.1	Newport Harbour, RI	USA	41.486 N 71.351 W	99.719	1426	0
FJ882054.1	North Sea, 2 m depth		54.420 N 6.480 E	99.717	1415	0
FJ154967.1	ocean water from Bohai Bay	China		99.93	1422	0
HM057661.1	ocean water from the Yellow Sea			99.93	1422	0
JQ712107.1	oil-contaminated seawater			100	1422	0
KJ094194.1	polluted marine sediments			99.848	1319	0
EF659446.1	Poole Harbour seawater			99.854	1371	0
HM591431.1	pretreatment systems for seawater reverse osmosis process	South Korea		99.789	1423	0
HM591461.1	pretreatment systems for seawater reverse osmosis process	South Korea		99.93	1423	0
JF514256.1	Seawater from coast of Xiaomaidao Island, Qingdao	China		99.79	1426	0
AM945553.1	sea water, Adriatic sea	Italy	44.69 N 12.52 E	99.774	1329	0
AM945571.1	sea water, Adriatic sea	Italy	44.69 N 12.52 E	100	1269	0
AM945577.1	sea water, Adriatic sea	Italy	44.69 N 12.52 E	99.924	1315	0
AM945578.1	sea water, Adriatic sea	Italy	44.69 N 12.52 E	99.925	1325	0
AM945580.1	sea water, Adriatic sea	Italy	44.69 N 12.52 E	99.774	1330	0

AM945584.1	sea water, Adriatic sea	Italy	44.69 N 12.52 E	99.696	1317	0
AM945585.1	sea water, Adriatic sea	Italy	44.69 N 12.52 E	99.699	1328	0
EU930869.3	sea water sample, Gwangyang Bay	South Korea		99.928	1385	0
AB496659.1	Seawater, Shizuoka, Shimoda, Oura Bay	Japan		100	1361	0
FJ425223.1	seawater			99.783	1384	0
FJ436731.1	seawater, coast of Qingdao	China	36.0276 N 120.1846 E	99.925	1328	0
JQ195110.1	seawater; next to dolphin A, San Diego Bay	USA: San Diego, CA		99.844	1281	0
JQ195194.1	seawater; next to dolphin A, San Diego Bay	USA: San Diego, CA		99.844	1283	0
JQ195765.1	seawater; next to dolphin C, San Diego Bay	USA: San Diego, CA		99.922	1281	0
JQ195767.1	seawater; next to dolphin C, San Diego Bay	USA: San Diego, CA		100	1281	0
JQ196782.1	seawater; next to dolphin E, San Diego Bay	USA: San Diego, CA		100	1281	0
JQ712031.1	surface seawater offshore Qingdao	China		99.367	1422	0
JQ712040.1	surface seawater offshore Qingdao			99.93	1422	0
JQ712053.1	surface seawater offshore Qingdao			99.93	1422	0
JQ712057.1	surface seawater offshore Qingdao			100	1422	0
NR_108333.1	coastal region of Qingdao	China	36.027N 120.184E	100	1370	0

Table S9: Environmental distribution of *Roseobacter* sp. GAI101, based on the 16S rRNA blast hits in the NR Blast databases from NCBI, with minimum 99.0% identity.

Blast hit Accession	Isolation source	Country	coordinates	% nucleotide Identity	Alignment length	evalue
JX529099.1	500m depth water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		47.9533 S 15.1437 W	99.561	1367	0

JX529112.1	500m depth water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		47.9533 S 15.1437 W	99.634	1367	0
JX529220.1	500m depth water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		47.9533 S 15.1437 W	99.561	1367	0
JX531118.1	500m depth water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		47.5015 S 15.4450 W	99.561	1367	0
JX531139.1	500m depth water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		47.5015 S 15.4450 W	99.635	1368	0
JX531386.1	500m depth water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		47.5015 S 15.4450 W	99.415	1368	0
AJ278782.1	Antarctic seawater isolate			99.571	1399	0
EU016167.1	Arctic deep-sea sediment			99.719	1424	0
FJ889522.1	Arctic Ocean			99.58	1430	0
FJ889535.1	Arctic Ocean			99.579	1424	0
FJ889536.1	Arctic Ocean			99.579	1424	0
FJ889527.1	Arctic Ocean			99.51	1430	0
FJ889528.1	Arctic Ocean			99.511	1431	0
FJ889530.1	Arctic Ocean			99.368	1424	0
FJ889531.1	Arctic Ocean			99.28	1388	0
FJ889542.1	Arctic Ocean			99.298	1424	0
EU365544.1	Arctic seawater			99.58	1430	0
EU365589.1	Arctic seawater			99.65	1430	0
JX525417.1	chlrophyll maxima (~30m) water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		48.0329 S 15.7856 W	99.561	1367	0

JX525429.1	chlrophyll maxima (~30m) water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		48.0329 S 15.7856 W	99.707	1367	0
JX525500.1	chlrophyll maxima (~30m) water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		48.0329 S 15.7856 W	99.489	1370	0
JX525579.1	chlrophyll maxima (~30m) water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		48.0329 S 15.7856 W	99.634	1367	0
JX527826.1	chlrophyll maxima (~30m) water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		47.9533 S 15.1437 W	99.488	1368	0
FN377731.1	clay-like sticky sediment with cylindrical plants/weeds on surface less than KB3	Svalbard, Kongsfjord region	78.59 N 11.56 E	99.52	1459	0
KJ939484.1	continental shelf sediments of Bay of Bengal			99.56	1364	0
NR_043547.1	East Sea	Korea		99.217	1404	0
FR684971.1	marine biome, fjord, coastal water	Norway, Raunefjord	60.27 N 5.22 E	99.508	1423	0
KJ475186.1	marine sediment, Ross Sea, Antarctica		76°06'17.10''S/ 169°12'45.36''E - 76°41'03.60"S/ 169°11'30.66"E	99.634	1365	0
AB733557.1	Nansei-Shotō Trench off Miyako Island, Okinawa Prefecture, southern Japan			99.719	1421	0
AY573043.1	Ny-Alesund, Svalbard, Norway	arctic	79° N, 12° E	99.579	1426	0
AY697915.1	seawater	Antarctica		99.37	1428	0
GQ358930.1	seawater from Kongsfjorden, Spitsbergen	Norway		99.579	1424	0
AF007257.2	seawater, Skidaway River			99.929	1414	0

AY794211.1	Soil from Ushuaia	Argentina	54°80' S and 68°13' W	99.37	1429	0
JX310158.1	Southern Ocean		47–48◦ South and 15–17◦ West	99.04	1458	0
GU584137.1	water sample	Antarctica	47–48°S and 15–17°W	99.588	1458	0
EF471650.1	whole surface water from Chesapeake Bay		39.13 N 76.33 W	99.719	1423	0
AY902203.1				99.489	1369	0
JX529304.1	500m depth water samples filtered on 0.22 micron filter paper; derived from the Southern ocean iron fertilization experiment (LOHAFEX)		47.9533 S 15.1437 W	99.122	1367	0

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Table S10: Environmental distribution of *Roseobacter* sp. SIO, based on the 16S rRNA blast hits in the NR Blast databases from NCBI, with minimum 99.6% identity.

Blast hit Accession	Isolation source	Country	coordinates	% nucleotide Identity	Alignment length	evalue
JX864948.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.692	325	1.92E-166
JX865037.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.692	325	1.92E-166
JX865038.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.692	325	1.92E-166
JX865039.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.692	325	1.92E-166
JX865040.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.692	325	1.92E-166
JX865041.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.692	325	1.92E-166
JX865044.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.688	320	1.16E-163
JX865047.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.692	325	1.92E-166
JX865055.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.692	325	1.92E-166
JX865056.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.692	325	1.92E-166
JX865060.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.692	325	1.92E-166
JX865061.1	5m depth, the Gulf of Trieste, northernAdriatic Sea, NE Mediterranean			99.692	325	1.92E-166
AB703474.1	coastal hot spring, Kagoshima, Ibusuki hot spring	Japan:		99.692	325	1.92E-166
AB703500.1	coastal hot spring, Kagoshima, Ibusuki hot spring	Japan:		99.692	325	1.92E-166

AF007250.1	coastal seawater, Sapelo Island			99.686	318	1.50E-162
LT549272.1	corrosion biofilm Boothbay Harbor	USA:Maine	43.8443 N 69.6409 W	99.692	325	1.92E-166
LT549313.1	corrosion biofilm Boothbay Harbor	USA:Maine	43.8443 N 69.6409 W	99.692	325	1.92E-166
LT549329.1	corrosion biofilm Boothbay Harbor	USA:Maine	43.8443 N 69.6409 W	99.692	325	1.92E-166
LT549343.1	corrosion biofilm Boothbay Harbor	USA:Maine	43.8443 N 69.6409 W	99.692	325	1.92E-166
DQ234090.2	Danshui river estuary, mangrove	Northern Taiwan		99.692	325	1.92E-166
DQ234137.2	Danshui river estuary, mangrove	Northern Taiwan		99.692	325	1.92E-166
DQ234157.2	Danshui river estuary, mangrove	Northern Taiwan		99.692	325	1.92E-166
DQ234162.2	Danshui river estuary, mangrove	Northern Taiwan		99.692	325	1.92E-166
DQ234224.2	Danshui river estuary, mangrove	Northern Taiwan		99.692	325	1.92E-166
KC006265.1	estuary in middle of Jiulong River	China	24.26 N 117.127 E	99.69	323	2.48E-165
KC006295.1	estuary in middle of Jiulong River	China	24.26 N 117.129 E	99.692	325	1.92E-166
KT275136.1	Gut of Olive Flounder Paralichthys olivaceus			99.692	325	1.92E-166
EF215736.1	inert artificial surfaces submerged in marine water on Qingdao coast	China		99.692	325	1.92E-166
AY712068.1	lon=81.2699W, lat=31.3929N; surface water collected on Jul 18, 2001, Sapelo Island Microbial Observatory Dean Creek Marsh sampling site	USA: Georgia		99.692	325	1.92E-166
AY937021.1	marine sediment Nagasaki	Japan:		99.692	325	1.92E-166
AY941090.1	marine sediment Nagasaki	Japan:		99.692	325	1.92E-166
KC462965.1	nature reserve Kullaberg	Sweden		99.692	325	1.92E-166
KC462968.1	nature reserve Kullaberg	Sweden		99.692	325	1.92E-166
EU799217.1	Newport Harbour, RI	USA	41.486 N 71.351 W	99.692	325	1.92E-166
EU799316.1	Newport Harbour, RI	USA	41.486 N 71.351 W	99.692	325	1.92E-166
EU799841.1	Newport Harbour, RI	USA	41.486 N 71.351 W	99.692	325	1.92E-166
JQ432672.1	Northern Adriatic Sea, NE Mediterranean			99.692	325	1.92E-166

KF185598.1	Northern Adriatic Sea: Gulf of Trieste,coastal seawater sample 10m depth			99.692	325	1.92E-166
AY576710.1	northwestern Mediterranean Sea			99.692	325	1.92E-166
GQ347802.1	Saanich Inlet, 10 m depth		48.5883 N 123.5037 W	99.692	325	1.92E-166
AF190747.1	Scripps Institution of Oceanography Pier			100	325	4.13E-168
U64005.1	Scripps Pier			99.692	325	1.92E-166
AM945581.1	sea water	Italy:Adriatic sea	44.69 N 12.52 E	99.692	325	1.92E-166
HQ203887.1	seawater			99.692	325	1.92E-166
HM921237.1	seawater, Kiel Fjord		54°27’4N, 10°12’E	99.692	325	1.92E-166
FJ161222.1	Shandong coast	China		99.69	323	2.48E-165
FJ161239.1	Shandong coast, China			99.692	325	1.92E-166
EF491274.1	steel surfaces immerged in marine waterof Qingdao Coast			99.692	325	1.92E-166
AB362414.1	surface seawater	Japan:Niigata, Urahama coast		99.692	325	1.92E-166
HE981601.1	surface seawater	Spain:Mallorca Island, Palma Harbour		99.688	321	3.21E-164
KP198349.1	surface seawater	USA: Cape Cod Bay	41.744 N 70.219 W	99.692	325	1.92E-166
GU061006.1	Yellow Sea intertidal beach	Korea		99.692	325	1.92E-166
GU061090.1	Yellow Sea intertidal beach	Korea		99.692	325	1.92E-166
GU061106.1	Yellow Sea intertidal beach	Korea		99.692	325	1.92E-166
AF365990.1		USA: off La Jolla, CA		99.692	325	1.92E-166
AY536559.1				99.692	325	1.92E-166
JN653206.1		USA: Bayboro Harbor, Florida	27.76 N 82.63 W	99.692	325	1.92E-166

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265 **Table S11: Prophage prediction using Phaster for the contigs containing the glutaredoxin gene**

Organism	Contig accession	Predicted prophage position	Prophage score	Glutaredoxin position	Glutaredoxin accession	Glutaredoxin associated to prophage
Rhizobium sp. Leaf383	NZ_LMQD01000008	1407-72420	intact	39353..39715	WP_082506598	yes
Sinorhizobium sp. LM21 (plasmid)	KM659098	11856-47482	questionable	26157..26360	AJW30150	yes
Hepatospora eriocheir strain GB1	LVKB01000119	none		3861..4241	ORD96177	
Hepatospora eriocheir strain GB2	LVKB01000120	none		3003..3317	ORD96175	

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268 **Table S12: Prophage prediction using Phaster for the contigs containing the RNR genes**

Organism	Contig accession	Predicted prophage position	Prophage score	RNR position	RNR accession	RNR associated to prophage
Aminobacter sp. J41	NZ_JAGL01000009.1	44041-86811	intact	72083-74071	WP_024847845	yes
Inquilinus limosus MP06	NZ_JANX01000001.1	32458-86185	questionable	47409..49382	WP_034830439	yes
Azorhizobium caulinodans ORS 571	NC_009937.1	4097072-4138015	questionable	4122652..4124646	WP_012172112	yes
Microvirga flocculans ATCC BAA-817	NZ_JAEA01000002.1	156454-179652	incomplete	163007..164998	WP_027314944	yes
Streptomyces sp. RTd22	NZ_CP015726.1	8923271-8946147	incomplete	8923015..8924970	WP_063728949.1	yes
Streptomyces sp. CB03238	NZ_NBCN01000010	231547-250436	incomplete	227661..229613	WP_084899922	in vicinity
Streptomyces sp. NRRL F-4489	NZ_LLZI01000124	13535-24084	incomplete	19443..21374	WP_066977110	yes
Streptomyces rimosus subsp. rimosus strain NRRL WC-3904	NZ_JOCQ01000004	101674-119267	incomplete	111002..112933	WP_050514495	yes

Solirubrobacter sp. URHD0082	NZ_AUEK01000007	344542-357687	incomplete	307203..309158	WP_051323998	in vicinity
Labrenzia alexandrii DFL-11	EQ973124	3254-38475	questionable	15955..18009	EEE42840	yes
Methylobacterium sp. Leaf361	NZ_LMPY01000023.1	581118-616465	questionable	594636..596663	WP_082557943	yes
Methylobacterium aquaticum plasmid pMaq22A_3p	NZ_AP014707	65911-85601	incomplete	83571..85601	WP_060851403	yes
Marinobacter similis strain A3d10	CP007151.1	3645543-3690844	intact	3653541..3655502	AHI29708.1	yes
Protochlamydia amoebophila strain EI2 DB44_AM	NZ_JSAN01000012.1	none		231..2243	WP_039355956	no
Gonium pectorale isolate NIES-2863	LSYV01000168	none		31484..37421	KXZ42284.1	no
Volvox carteri f. nagariensis	NZ_JOCQ01000004.1	none		<195692..>202991	XP_002955713	no
Criblamydia sequanensis	NZ_CCEJ010000007	none		134416..136428	WP_041017866	no
Parachlamydia acanthamoebae strain Bn9	NZ_BAWW01000003	none		161269..163278	WP_006342605	no
Parachlamydia acanthamoebae strain OEW1 DB43_DW	NZ_JSAM01000017	none		15051..17060	WP_006342605	no
Parachlamydia acanthamoebae UV7	NC_015702	none		895276..897285	WP_006342605	no
Parachlamydia acanthamoebae str. Hall's coccus	NZ_ACZE01000088	none		17369..19378	WP_006342605	no
Estrella lausannensis	CWGJ01000019	none		124560..126572	CRX38802	no
Candidatus Rubidus massiliensis	CCSC01000001	1811588-1828589	incomplete	1412888..1414906	CDZ80757	no
Waddlia chondrophila	NC_014225	none		368424..370439	WP_013181434	no
Waddlia chondrophila 2032/99	FR872643	none		26560..28614	CCB90950	no

Neochlamydia sp. TUME1	NZ_JRXI01000032	none		32033..34045	WP_039383590	no
Neochlamydia sp. S13	NZ_BASK01001259	none		36271..38283	WP_042242257	no
Neochlamydia sp. EPS4	NZ_JSDQ01000098	none		4026..6038	WP_044882767	no
Parachlamydia sp. C2	NZ_FCNU01000032.1	none		238258..240270	WP_068471381	no
Candidatus Protochlamydia amoebophila UWE25	NC_005861	none		178729..180741	WP_011174661	no
Candidatus Protochlamydia sp. W-9	NZ_BCPZ01000156.1	none		50658..52670	WP_075883166	no
Candidatus Protochlamydia sp. R18	NZ_BASL01000738	none		1527..3539	WP_042281843	no

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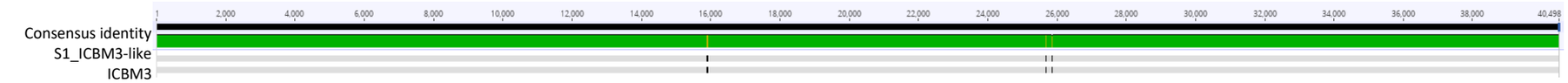
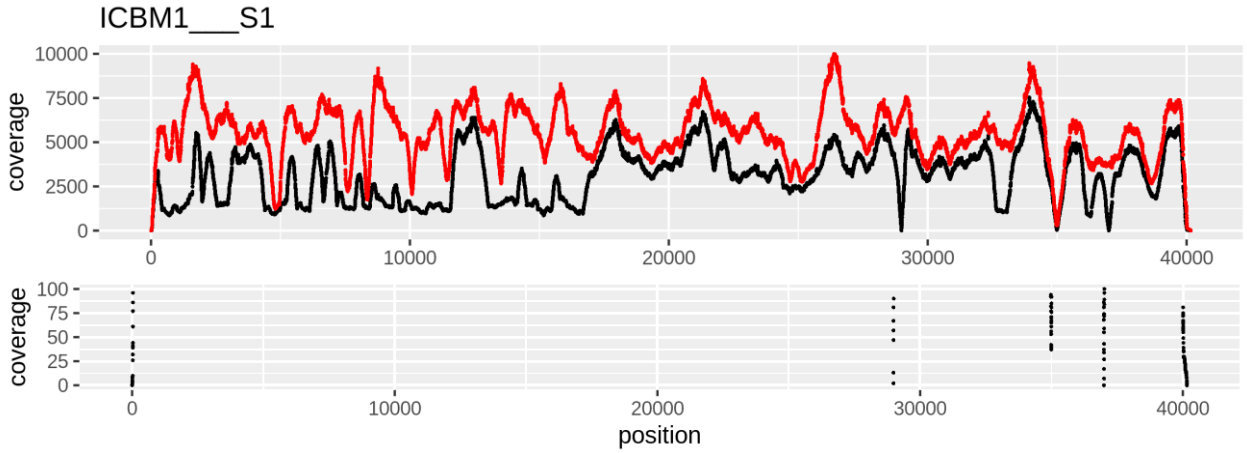
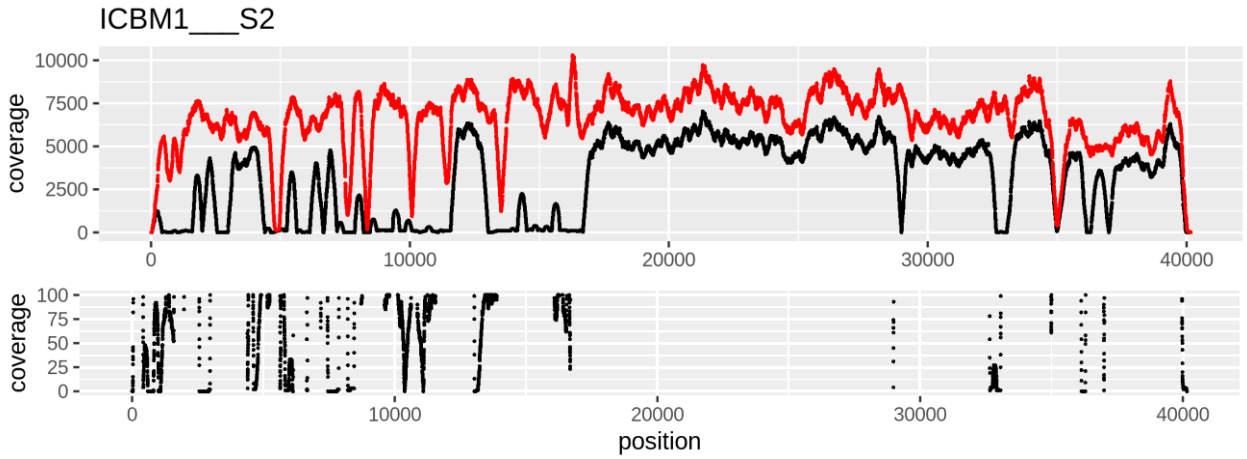
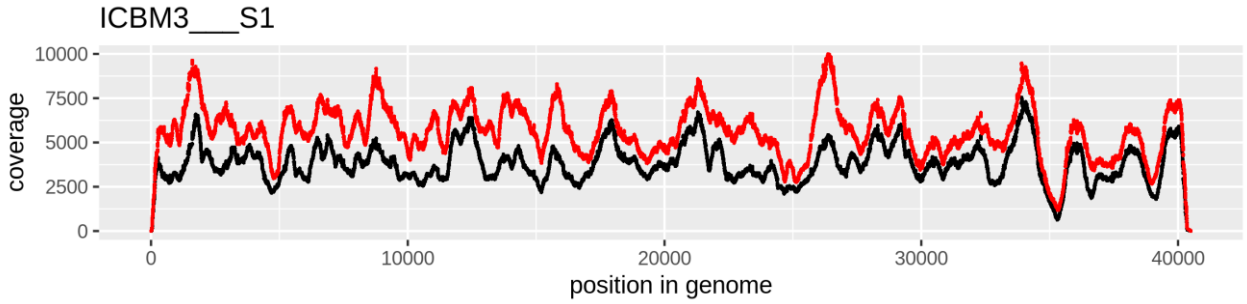
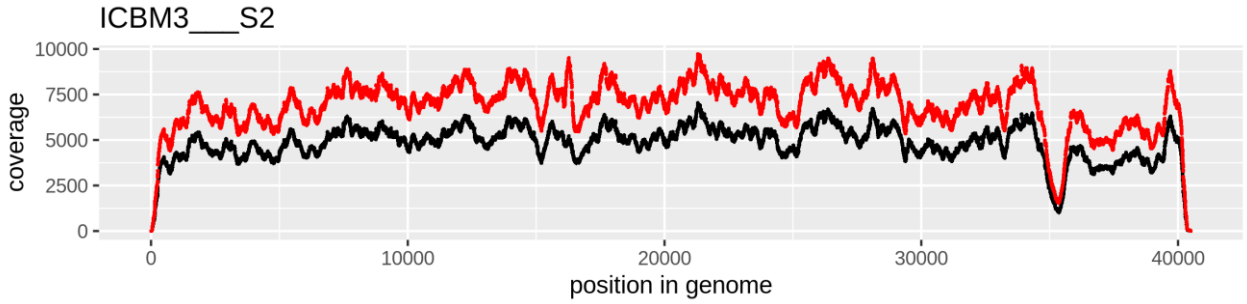


Figure S1: Pairwise genome comparison between ICBM3 and the ICBM3-like genome assembled from the S1 enrichment. In the alignment field, the differences are marked in black. In the consensus identity field, the presence of mismatches is signaled in orange.


ICBM1___S1
coverage
10000
7500
5000
2500
0
0
10000
20000
30000
40000
coverage
100
75
50
25
0
0
10000
20000
30000
40000
position


ICBM1___S2
coverage
10000
7500
5000
2500
0
0
10000
20000
30000
40000
coverage
100
75
50
25
0
0
10000
20000
30000
40000
position


ICBM3___S1
coverage
10000
7500
5000
2500
0
0
10000
20000
30000
40000
position in genome


ICBM3___S2
coverage
10000
7500
5000
2500
0
0
10000
20000
30000
40000
position in genome

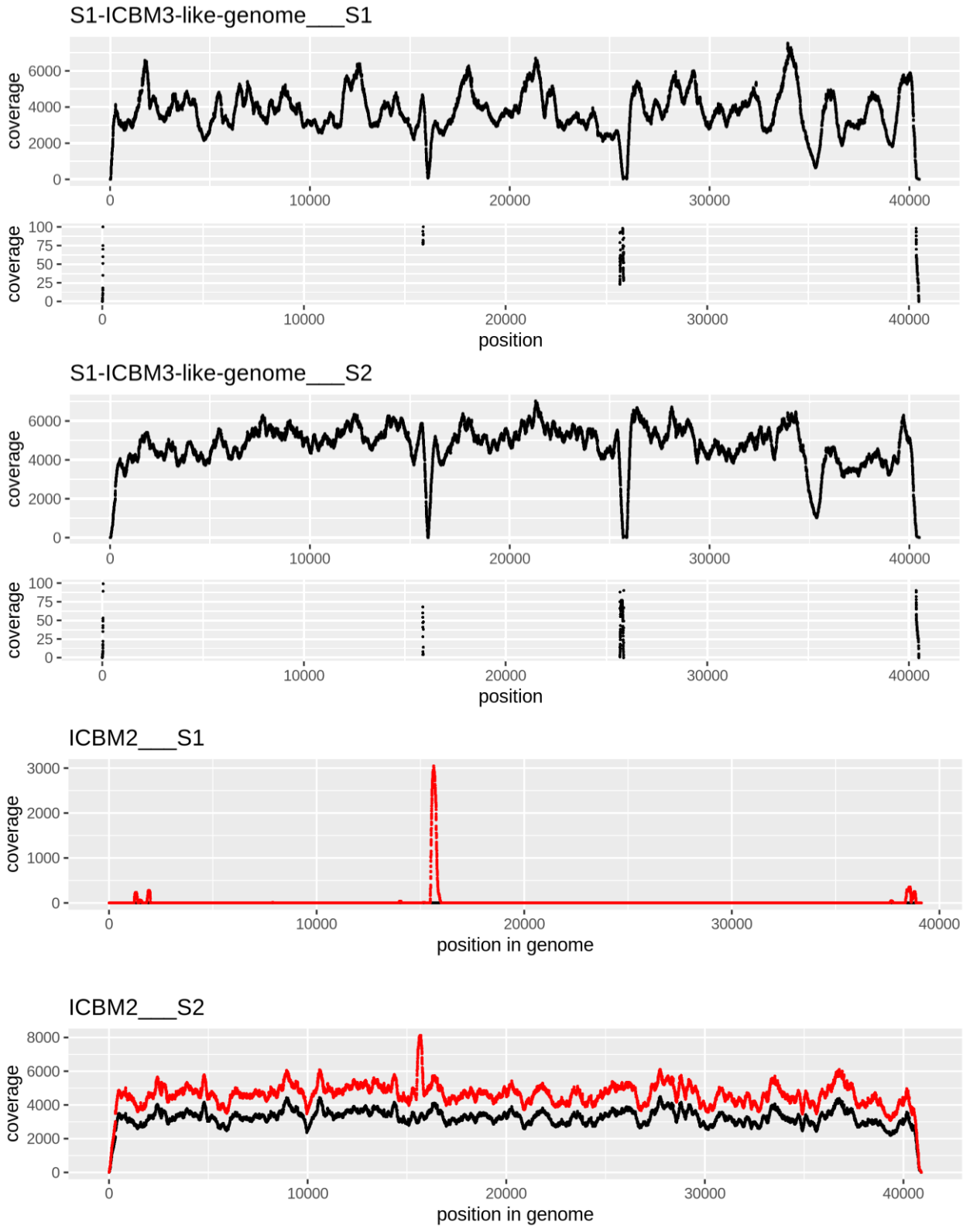


Figure S2: Plots showing the read coverage along the phage genomes for the S1 and S2 phage enrichments. In black – coverage of reads with 100% identity. In red – coverage of reads with >95% identity. The small differences between ICBM3 and the ICBM3-like genome from S1 enrichment could be due to sequencing errors, as the coverage in the variable regions dropped sharply for the genome retrieved from S1, but not for ICBM3 (see Figure S1).

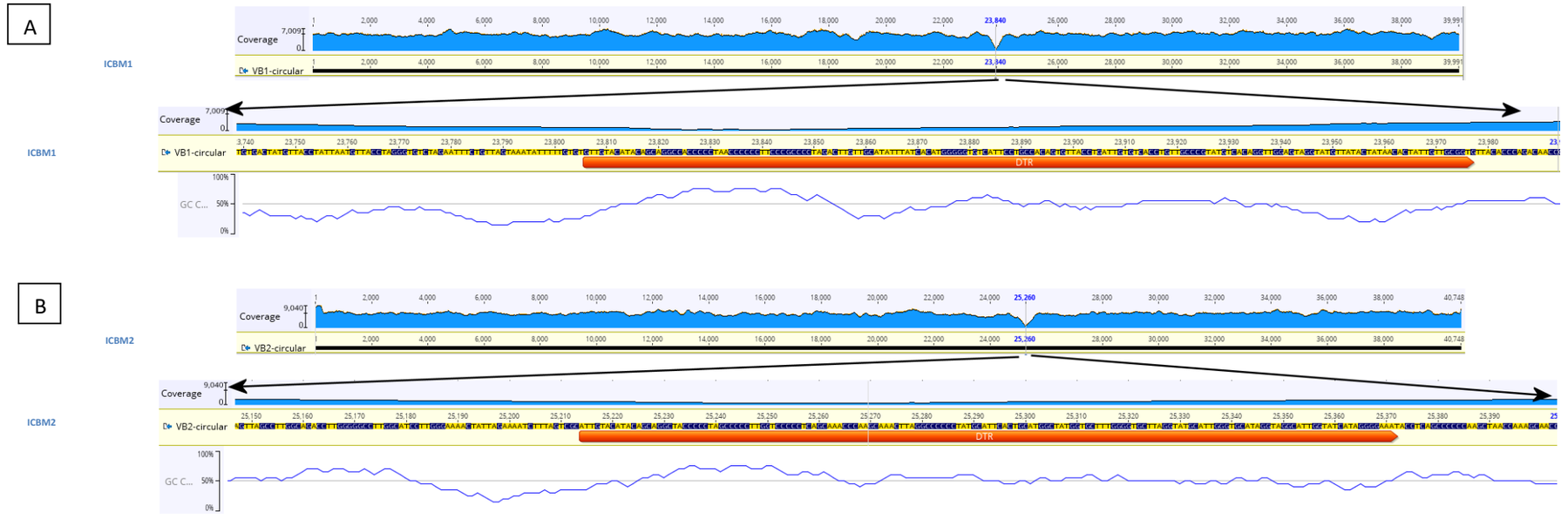


Figure S3: Illumina short read sequencing, distribution of reads along the assembled contigs for ICBM1 (A) and ICBM2 (B) phages. The position of the drop in coverage is indicated in blue numbers. The magnifications show the region corresponding to the drop in coverage, with the corresponding %GC graph (blue line) and the DTR positions (as determined from PacBio read coverage) marked in red.

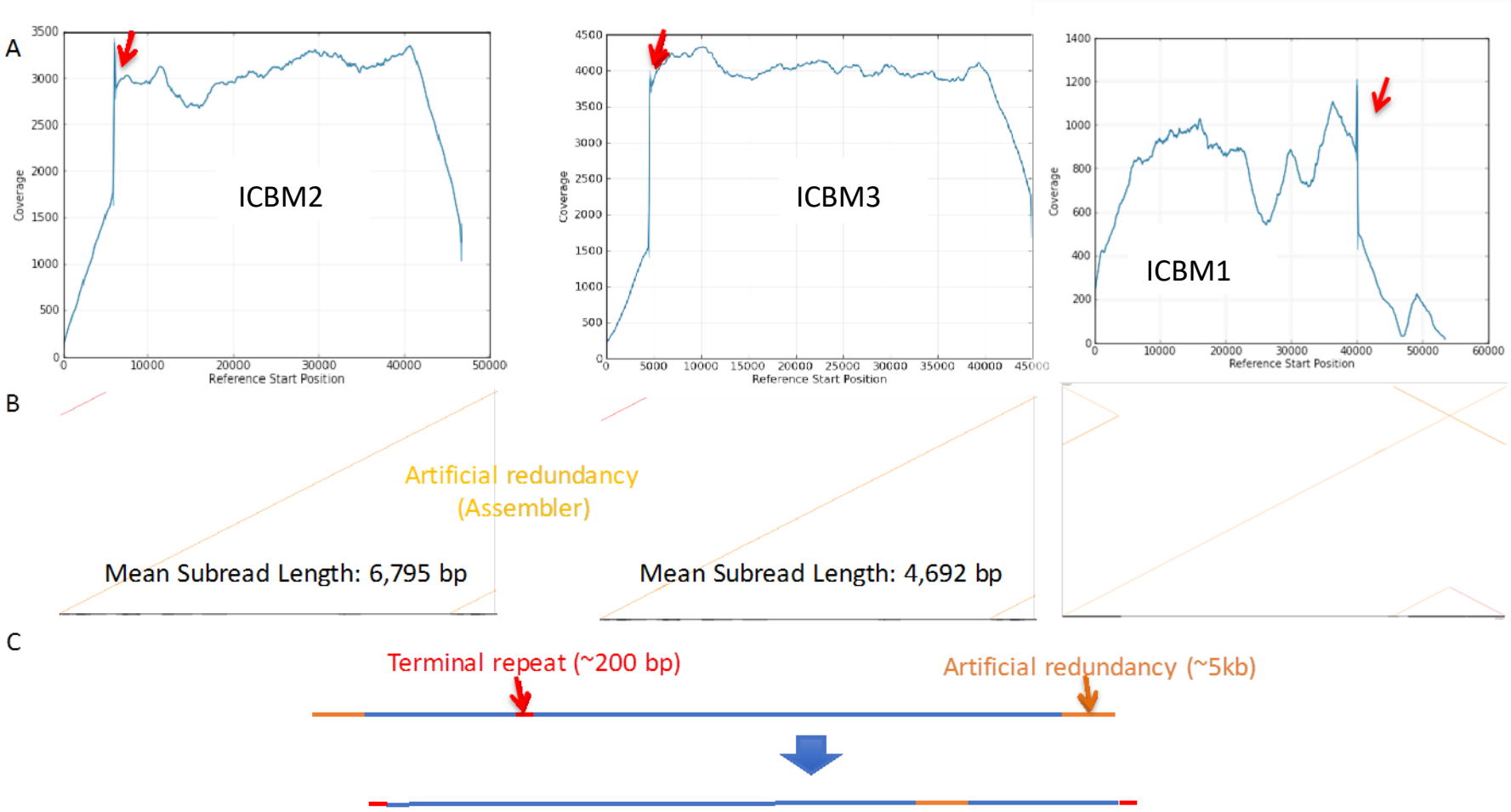


Figure S4. A. Coverage plots (window = 100 bp) of long read genome assemblies of both phages ICBM1 and ICBM2 contained within enrichment. Distinct coverage spikes of about 100 bp can be observed at position 5kb (terminal redundancies, red arrows).
B. Artificial redundancies at the end of the contigs (shown here as dotplots) are produced by the genome assembler usually at the size of the mean subread length suggesting a circular genome structure.

C. Delineation of the final phage genome structure applying the “linearization” process. The artificial redundancy (yellow) is removed and the terminal repeat (red) placed twice at the end of the phage genomes

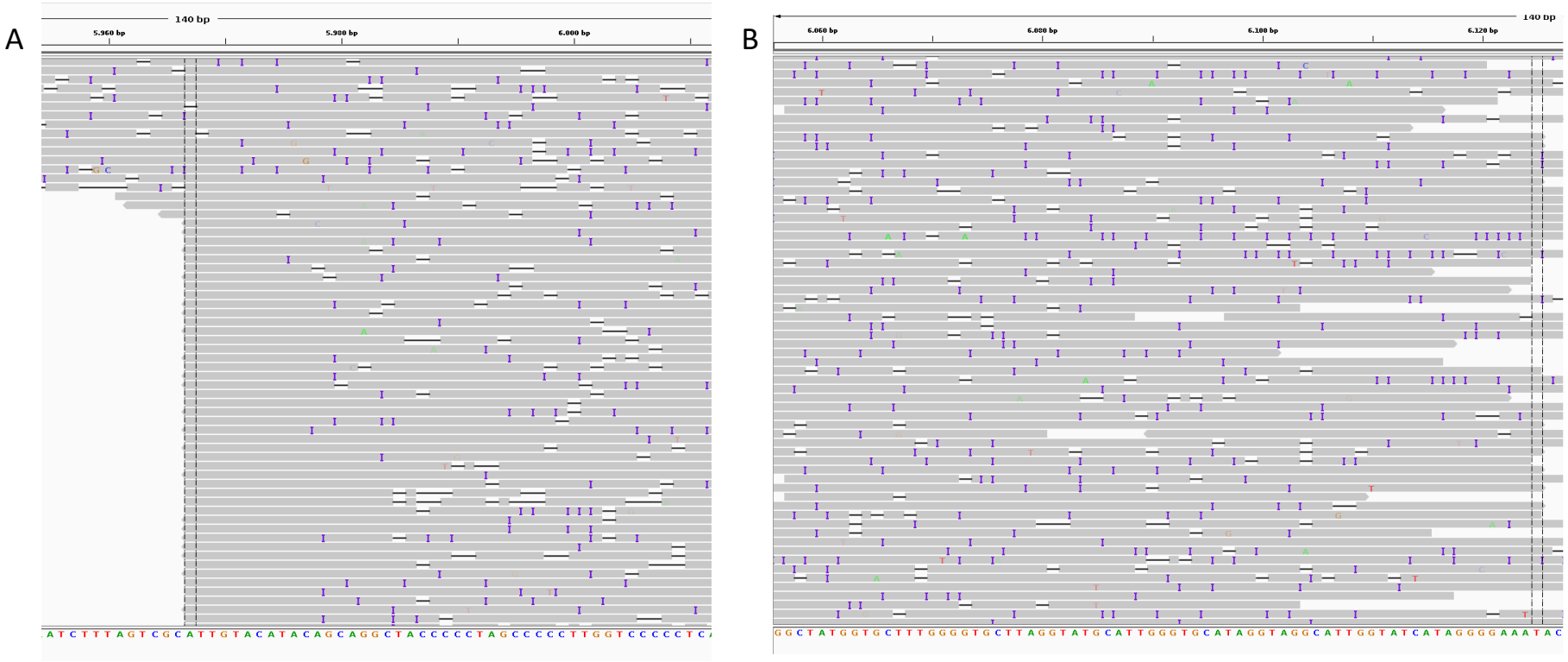


Figure S5. Distinct start (A) and end positions (B) of a linear phage genome as retrieved from a visualization of a long read genome mapping by IGV.

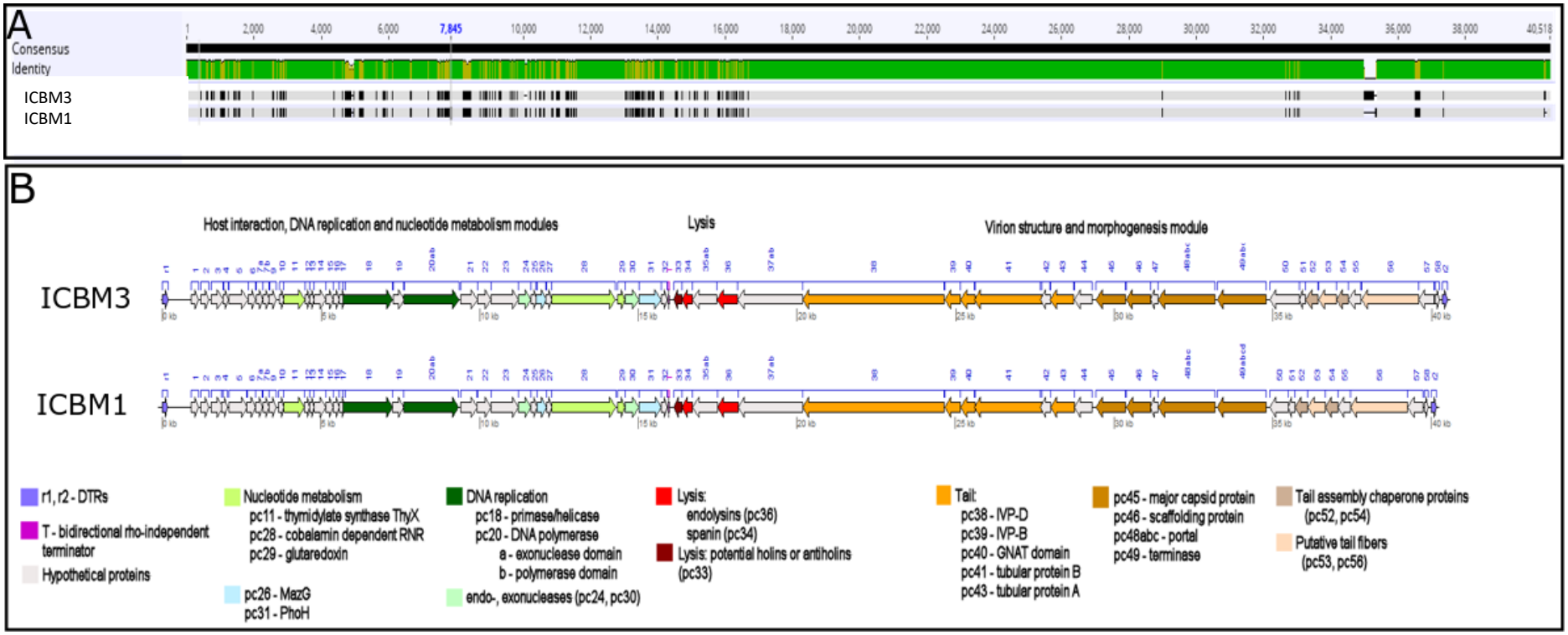
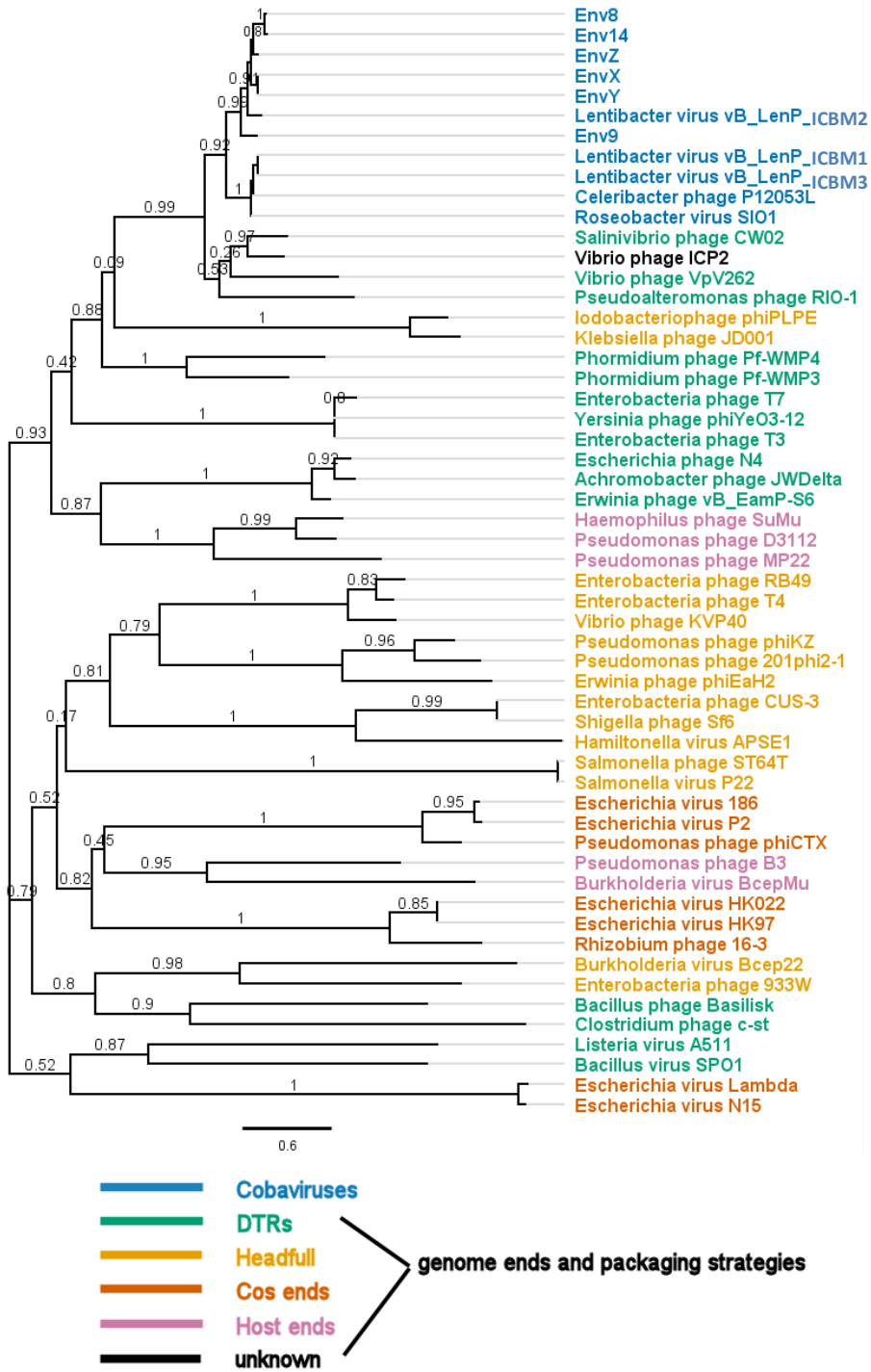


Figure S6: Genome comparison of ICBM1 and ICBM3 phages: A) showing the location of mismatches and deletions and B) showing the predicted genes.



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318 **Figure S7: Phylogenetic analysis of the Terminase large subunit from cobaviruses and other phages**
319 **with known genome ends and packaging strategies. The evolutionary history was inferred using the**
320 **approximately-maximum-likelihood method implemented in FastTree 2.1.5. The node labels**
321 **represent Fast Tree support values. The tree is drawn to scale, with branch lengths measured in**
322 **number of substitutions per site. The tree is unrooted.**

323

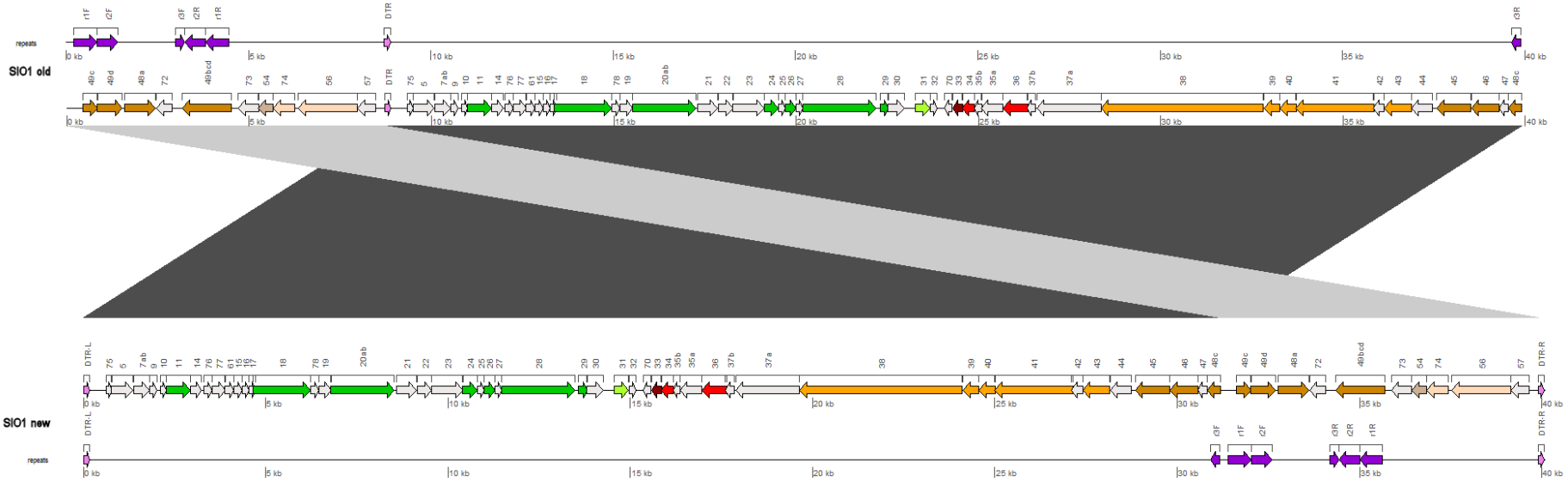

Figure S8: Genomic maps of the SIO1 phage, before and after genome end correction. Purple arrows: position of the inverted repeats. Pink arrows: position of DTRs.

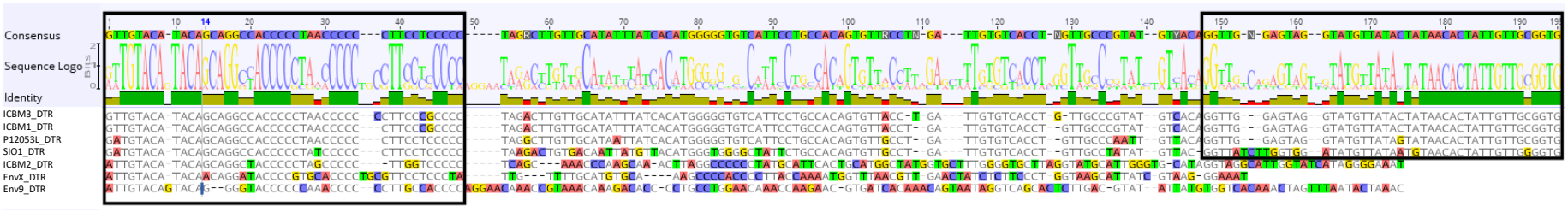

Figure S9: Multiple alignments of genomic ends from all cobaviruses with complete genome.

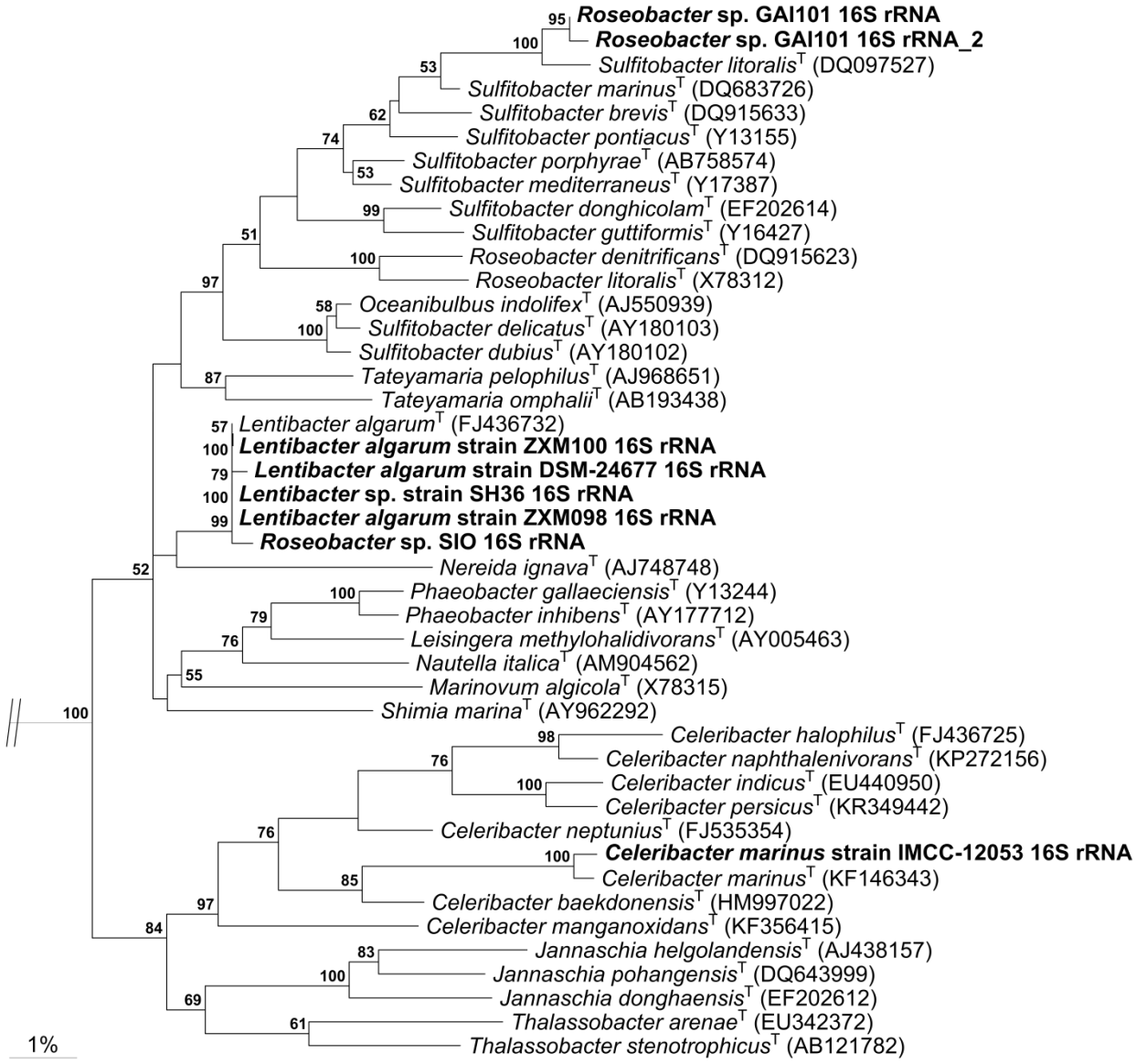

Figure S10: Neighbour joining tree based on 16S rRNA gene similarity showing the phylogenetic affiliation of bacterial hosts analysed in this study (bold) within the *Rhodobacteraceae*. Sequences of type material (>1300bp) were used to construct the backbone tree. Only bootstrap values ≥50% (derived from 1500 replicates) are shown. Selected sequences related to *Gammaproteobacteria* were used as outgroup to define the root of the tree (not shown). GenBank accession numbers are given in parentheses. Scale bar indicates percentage of sequence divergence.

Figure S11: Phylogenetic positioning of the Cobaviruses (green font) and spanin containing prophages from *Rhodobacteraceae* (blue font). The whole-genome-based phylogeny was inferred using the Genome-BLAST Distance Phylogeny method implemented in the VICTOR web service, using the amino acid data.

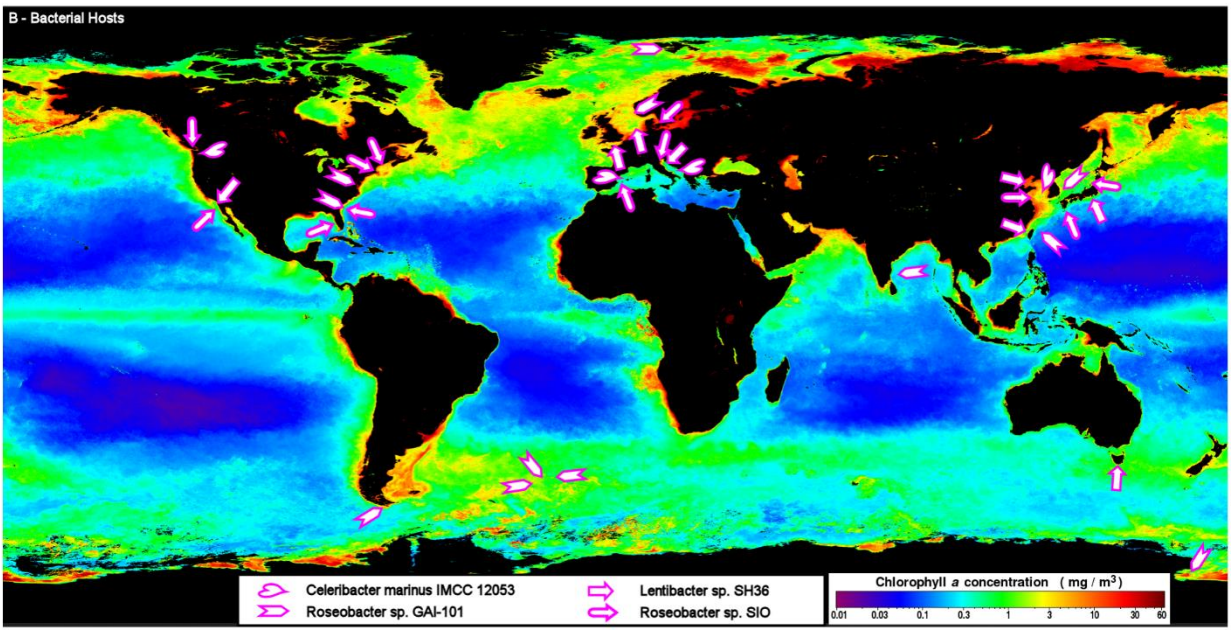

Figure S12: Locations in which cobavirus hosts were found based on a 16S rRNA survey in the NR Blast database and in the Tara Ocean samples.

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