Metagenomics analysis of bacteriophages and antimicrobial resistance from global urban sewage

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Supplementary Table 1

Coverage and depth of coverage distributions

		NCBI phages	IMG/VR genome	Huge phages
Coverage (%)	**Mean (SD)**	1.21 (4.96)	2.5 (7.78)	2.09 (8.09)
	Min;Max	0;96.08	0;97.62	0;97.25
Depth of coverage	**Mean (SD)**	0.03 (0.34)	0.07 (0.72)	0.18 (2.83)
	Min;Max	0;33.28	0;190.27[1]	0;176.58[2]

[1] The phage from IMG/VR genome having a depth of 190.27 is X3300014750_____Ga0134554_100011 with a sequence length of 103,348 bp. The phage was found in various datasets with coverages ranging between 3.35% to 93.55%

[2] The phage from custom huge phages having a depth of 176.58 is F27_PHAGE_CIR_34_39 with a sequence length of 200,395 bp. The phage was found in various datasets with coverages ranging between 0% to 59.14%.

Supplementary Table 2

Number of samples and phages within each region

	Total	AF	AS	EU	ME	NA	OC	SA
Number of samples	78	12	10	31	3	14	3	5
Number of phages								
NCBI	638	447 (70.1%)	454 (71.2%)	373 (58.5%)	178 (27.9%)	313 (49.1%)	129 (20.2%)	382 (59.9%)
IMG/VR all	54893	21392 (39.0%)	19794 (36.1%)	32157 (58.6%)	10461 (19.1%)	26354 (48.0%)	8847 (16.1%)	15834 (28.8%)
IMG/VR genomes	3699	2287 (61.8%)	2182 (59.0%)	2767 (74.8%)	1281 (34.6%)	2436 (65.9%)	993 (26.8%)	1881 (50.9%)
Huge phages	114	60 (52.6%)	81 (71.1%)	64 (56.1%)	32 (28.1%)	31 (27.2%)	13 (11.4%)	56 (49.1%)

AF, Africa; AS, Asia; EU, Europe; NA, North America; ME, Middle East, OC, Oceania; SA South America

Supplementary Table 3

Distribution of countries within income classes

Income class	AF	AS	EU	NA	ME	OC	SA	Total
High	0	1	23	14	0	3	0	41 (52.6%)
Middle	8	8	8	0	3	0	5	32 (41.0%)
Low	4	1	0	0	0	0	0	5 (6.4%)

For classification of countries please refer to Supplementary Table 1. Percentages are given as the percent of total samples.
AF, Africa; AS, Asia; EU, Europe; NA, North America; ME, Middle East; OC, Oceania; SA, South America

Supplementary Table 4

Summary of pooled datapoints within phage families and clades

Database	Order/Clade	Family	Number of datapoints (phages)
NCBI virus	*Caudovirales*	*Siphoviridae*	262
	Caudovirales	*Myoviridae*	173
	Caudovirales	*Autographiviridae*	74
	Caudovirales	*Podoviridae*	66
	Caudovirales	*Drexlerviridae*	17
	Tubulavirales	*Inoviridae*	11
	Petitvirales	*Microviridae*	10
	Caudovirales	*Demerecviridae*	10
	Caudovirales	*Herelleviridae*	6
	Caudovirales	*Ackermannviridae*	5
	Unclassified	Unclassified[†]	2
	Unclassified	*Plasmaviridae*	1
	Kalamavirales	*Tectiviridae*	1
Custom huge phages	Unknown		80
	Mahaphage		9
	Kabirphage		6
	Close to Whopperphage		6
	Whopperphage		5
	Jabbarphage		4
	Kaempephage		2
	Distinct group		2
	Enormephage		1

[†] The two unclassified phages were that of Enterobacteria phage P4 (NC_001609) and Hydrogenobaculum phage strain HP1 (NC_028651)

Supplementary Table 5

Procrustes correlation

	Correlation coefficient	**p-value**
NCBI phages & bacteria	0.8148	0.001
IMG/VR phages & bacteria	0.8956	0.001
Custom huge phages & bacteria	0.7054	0.001
Bacteria & ARGs	0.8813	0.001
NCBI phages & ARGs	0.8488	0.001
IMG/VR phages & ARGs	0.8956	0.001
Custom huge phages & ARGs	0.7379	0.001
NCBI other viruses & bacteria	0.7167	0.001
NCBI other viruses & ARGs	0.7619	0.001
IMG/VR other viruses & bacteria	0.8896	0.001
IMG/VR other viruses & ARGs	0.9033	0.001

Supplementary Table 6

Overview of sample annotations and temperatures at day of sample collection

Sample	Sample accession	Country	City	Region	Income class	Collection date	Temperature (°C)		
							Sample	Transport	Storage
ALB-17	SAMEA4527599	Albania	Tirana	EU	Upper middle	02 January	12	12	-73
AUS-18	SAMEA4527600	Australia	Woden	OC	High	02 April	19.3	4	-80
AUS-18a	SAMEA4527601	Australia	Melbourne	OC	High	02 August	23.8	4	-80
AUT-70	SAMEA4527602	Austria	Vienna	EU	High	16 February	7	18	-80
BGR-66	SAMEA4527603	Bulgaria	Sofia	EU	Upper middle	15 February	5	4	-70
BRA-53	SAMEA4527604	Brazil	Belém	SA	Upper middle	16 February	NR	4	-70
BRA-53a	SAMEA4527605	Brazil	Belém	SA	Upper middle	15 February	28	4	-80
BWA-19	SAMEA4527606	Botswana	Palapye	AF	Upper middle	25 January	32	4	-80
CAN-22	SAMEA4527607	Canada	Regina	NA	High	26 January	-5.6	4	-80
CAN-22a	SAMEA4527608	Canada	Calgary	NA	High	02 January	-4	4	-80
CAN-22b	SAMEA4527609	Canada	Toronto	NA	High	02 January	4	4	-80
CAN-22c	SAMEA4527610	Canada	Ottawa	NA	High	26 January	-0.7	4	-80
CHE-67	SAMEA4527611	Switzerland	Liebefeld	EU	High	27 January	3.8	-20	-70
CHN-64	SAMEA4527612	China	Guangzhou	AS	Upper middle	28 January	13	13	-70
CIV-13	SAMEA4527613	Côte d’Ivoire	Abidjan	AF	Lower middle	25 January	NR	5	-80
CZE-23	SAMEA4527615	Czechia	Brno	EU	High	02 January	11	9	-75
DEU-27	SAMEA4527616	Germany	Berlin	EU	High	02 August	NR	NR	-80
DNK-71-RA	SAMEA4527617	Denmark	Avedøre	EU	High	03 February	4	-20	10
DNK-71-RD	SAMEA4527618	Denmark	Damhusåen	EU	High	03 February	4	-20	10
DNK-71-RL	SAMEA4527619	Denmark	Lynetten	EU	High	03 February	4	-20	10
ECU-14	SAMEA4527620	Ecuador	Quito	SA	Upper middle	02 January	24	15	-80
ECU-14a	SAMEA4527621	Ecuador	Galápagos	SA	Upper middle	02 June	28	28	-80
ESP-75	SAMEA4527622	Spain	Barcelona	EU	High	02 October	14.5	10	-75
ETH-24	SAMEA4527623	Ethiopia	Addis Ababa	AF	Low	02 January	27	4	-80
FIN-25	SAMEA4527624	Finland	Helsinki	EU	High	26 January	-1	6	-80
GEO-59	SAMEA4527625	Georgia	Tbilisi	EU	Lower middle	25 January	1	4	-80

Sample	Sample accession	Country	City	Region	Income class	Collection date	Temperature (°C)		
							Sample	Transport	Storage
GHA-4	SAMEA4527626	Ghana	Tamale	AF	Lower middle	30 January	30	4	-20
HRV-68	SAMEA4527628	Croatia	Zagreb	EU	Upper Middle	02 January	14	42	-82
HUN-61	SAMEA4527629	Hungary	Budapest	EU	High	02 February	11	10	-80
IND-11	SAMEA4527630	India	Cochin	AS	Lower middle	27 January	32	15	-80
IRL-69	SAMEA4527631	Ireland	Dublin	EU	High	02 October	8	4	-80
IRN-12	SAMEA4527632	Iran	Tehran	ME	Upper middle	02 February	11	4	-80
ISL-28	SAMEA4527633	Iceland	Reykjavik	EU	High	02 February	-3	4	-77
ISR-29	SAMEA4527634	Israel	Jerusalem	ME	High	31 January	3	4	-80
ITA-30	SAMEA4527635	Italy	Rome	EU	High	02 February	13	4	-80
KAZ-6	SAMEA4527636	Kazakhstan	Almaty	AS	Upper middle	02 February	5	10	-80
KEN-72	SAMEA4527637	Kenya	Thika	AF	Lower middle	02 August	25	10	-80
KHM-21	SAMEA4527638	Cambodia	Phnom Penh	AS	Lower middle	02 May	34	34	-80
LKA-40	SAMEA4527639	Sri Lanka	Colombo	AS	Lower middle	28 February	31	30	-80
LUX-32	SAMEA4527640	Luxembourg	Belvaux	EU	High	02 January	9	6	-80
LVA-31	SAMEA4527641	Latvia	Riga	EU	High	02 January	3	4	-80
MDA-65	SAMEA4527642	Moldova	Chișinău	EU	Lower middle	04 February	NR	NR	NR
MKD-62	SAMEA4527643	Macedonia	Skopje	EU	Upper middle	02 February	19	4	-80
MLT-63	SAMEA4527644	Malta	Saint Venera	EU	High	02 January	17	10	-80
MYS-54	SAMEA4527645	Malaysia	Kuala Lumpur	AS	Upper middle	02 April	29	4	-80
NGA-50	SAMEA4527646	Nigeria	Lagos	AF	Lower middle	02 February	NR	4	-80
NLD-43	SAMEA4527647	Netherlands	Bilthoven	EU	High	27 January	11	5	-50
NOR-34	SAMEA4527648	Norway	Oslo	EU	High	02 March	1	4	-70
NPL-33	SAMEA4527649	Nepal	Kathmandu	AS	Low	30 January	26	26	-80
NZL-56	SAMEA4527650	New Zealand	Dunedin	OC	High	27 January	14	4	-80
PAK-7	SAMEA4527652	Pakistan	Karachi	AS	Lower middle	27 January	24	25	-80
PER-35	SAMEA4527653	Peru	Lima	SA	Upper middle	02 April	29	4	-80
POL-36	SAMEA4527651	Poland	Puławy	EU	High	02 February	8	4	-80
SEN-8	SAMEA4527654	Senegal	Dakar	AF	Low	02 February	NR	7	-80
SGP-52	SAMEA4527655	Singapore	Singapore	AS	High	02 March	31	30	-80
SRB-37	SAMEA4527656	Serbia	Belgrade	EU	Upper middle	27 January	10	4	-80
SVK-9	SAMEA4527657	Slovakia	Bratislava	EU	High	02 February	10	10	-80
SVN-38	SAMEA4527658	Slovenia	Ljubljana	EU	High	25 January	0	6	-70

Sample	Sample accession	Country	City	Region	Income class	Collection date	Temperature (°C)		
							Sample	Transport	Storage
SWE-41	SAMEA4527659	Sweden	Uppsala	EU	High	02 February	5	5	-75
SWE-41a	SAMEA4527660	Sweden	Gothenburg	EU	High	02 November	3	15	-76
TGO-44	SAMEA4527662	Togo	Lomé	AF	Low	26 January	30	30	-80
TUR-46	SAMEA4527663	Turkey	Ankara	ME	Upper middle	29 January	12	6.1	-83
TZA-15	SAMEA4527664	Tanzania	Moshi	AF	Low	02 March	27	23	-80
USA-74	SAMEA4527665	USA, Georgia	Atlanta	NA	High	02 February	24.4	4	-25
USA-74a	SAMEA4527666	USA, Washington	Seattle	NA	High	02 January	7.8	4	-80
USA-74b	SAMEA4527667	USA, Illinois	Chicago	NA	High	24 February	3.9	5	-80
USA-74c	SAMEA4527668	USA, Texas	El Paso	NA	High	23 February	16.1	4	6
USA-74d	SAMEA4527669	USA, Oregon	Portland	NA	High	02 October	15.5	NR	4
USA-74e	SAMEA4527670	USA, Texas	El Paso	NA	High	22 February	16.1	4	6
USA-74f	SAMEA4527671	USA, Texas	El Paso	NA	High	22 February	16.1	4	6
USA-74g	SAMEA4527672	USA, Texas	El Paso	NA	High	23 February	16.1	4	6
USA-74h	SAMEA4527673	USA, Colorado	Adams County	NA	High	NR	NR	NR	NR
USA-74i	SAMEA4527674	USA, Colorado	Boulder	NA	High	28 March	13.9	4	4
VNM-48	SAMEA4527675	Vietnam	Ho Chi Minh	AS	Lower middle	27 January	NR	NR	NR
XK-60	SAMEA4527676	Kosovo	Pristina	EU	Lower middle	02 March	11	6	-80
ZAF-39	SAMEA4527677	South Africa	Johannesburg	AF	Upper middle	02 April	30	4	-70
ZMB-49	SAMEA4527678	Zambia	Lusaka	AF	Lower middle	25 January	26.7	4	4
ZMB-49b	SAMEA4527679	Zambia	Kitwe	AF	Lower middle	01 December	27	4	4

Sample: Temperature of sample at time of sampling.

NR: Not reported; AF, Africa; AS, Asia; EU, Europe; ME, Middle East; NA, North America; OC, Oceania; SA, South America.

Supplementary Table 7

Phage family composition within the NCBI database[†]

Order	Family	Number of phage members
Caudovirales	*Ackermannviridae*	23
	Autographiviridae	349
	Chaseviridae	7
	Demerecviridae	55
	Drexlerviridae	71
	Myoviridae	623
	Podoviridae	241
	Siphoviridae	1410
	Herelleviridae	120
	Unclassified	2
Kalamavirales	*Tectiviridae*	7
Microvirales	*Microviridae*	61
Tubulavirales	*Inoviridae*	43
Vinavirales	*Corticoviridae*	2
Unclassified	*Plasmaviridae*	1

[†] Numbers as of June 2020. As the NCBI database utilised for mapping within the study is from March 2019, the numbers are likely less than the ones reported here; it was not possible to obtain a data on the database composition.

Supplementary Table 8

Quantile breaks and their corresponding percentages

Database	Quantile breaks	Colour representation[†]
NCBI phages	200	0.5%
IMG/VR genomes	15	6.7%
Huge phages	30	3.3%
NCBI families	10	10%
Clades of huge phages	30	3.3%

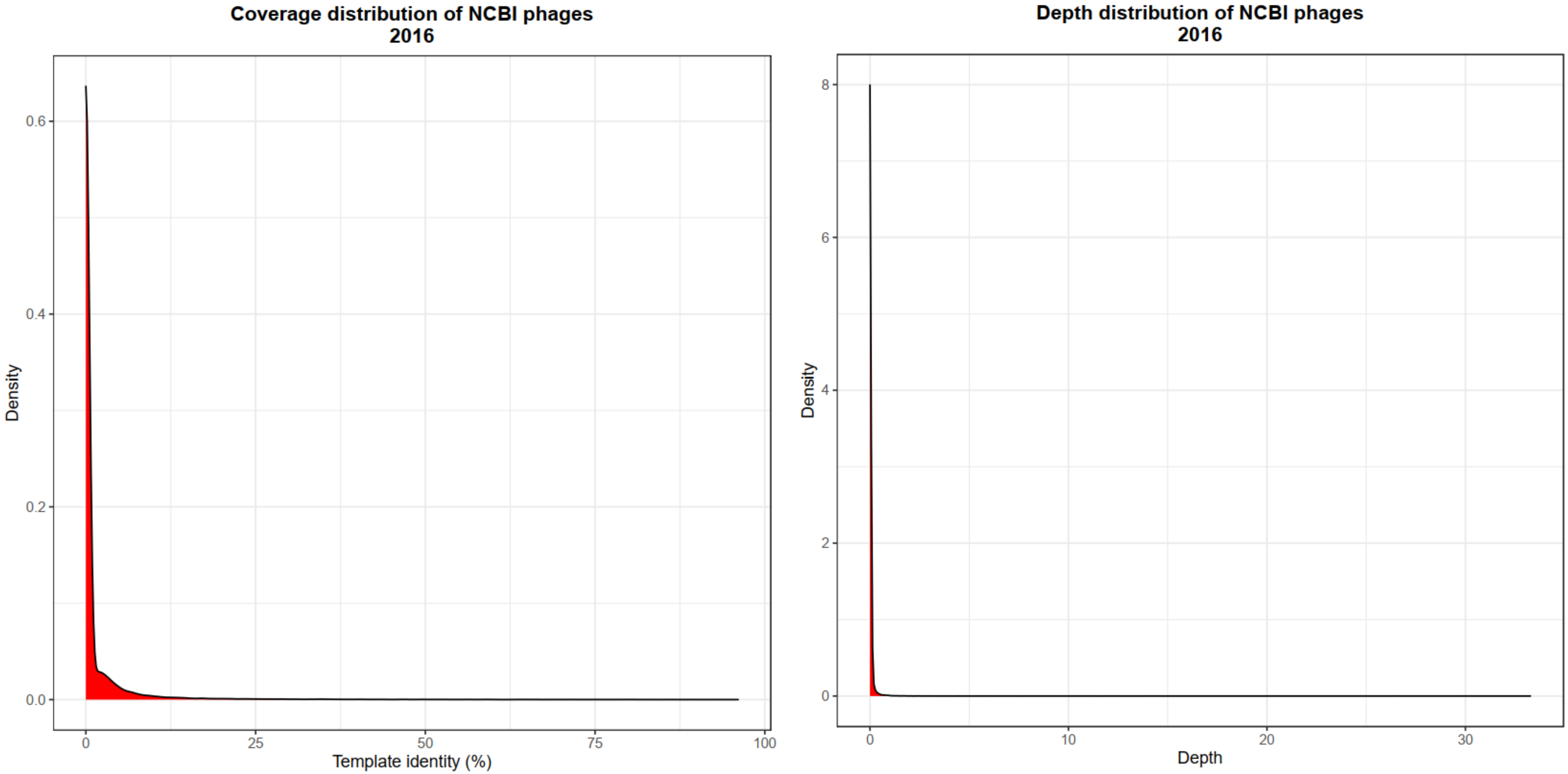
[†] Percentage of datapoints in the distribution represented by a single colour, e.g. the top 0.5% of the datapoints for NCBI phages are represented by pale yellow.

Supplementary Table 9

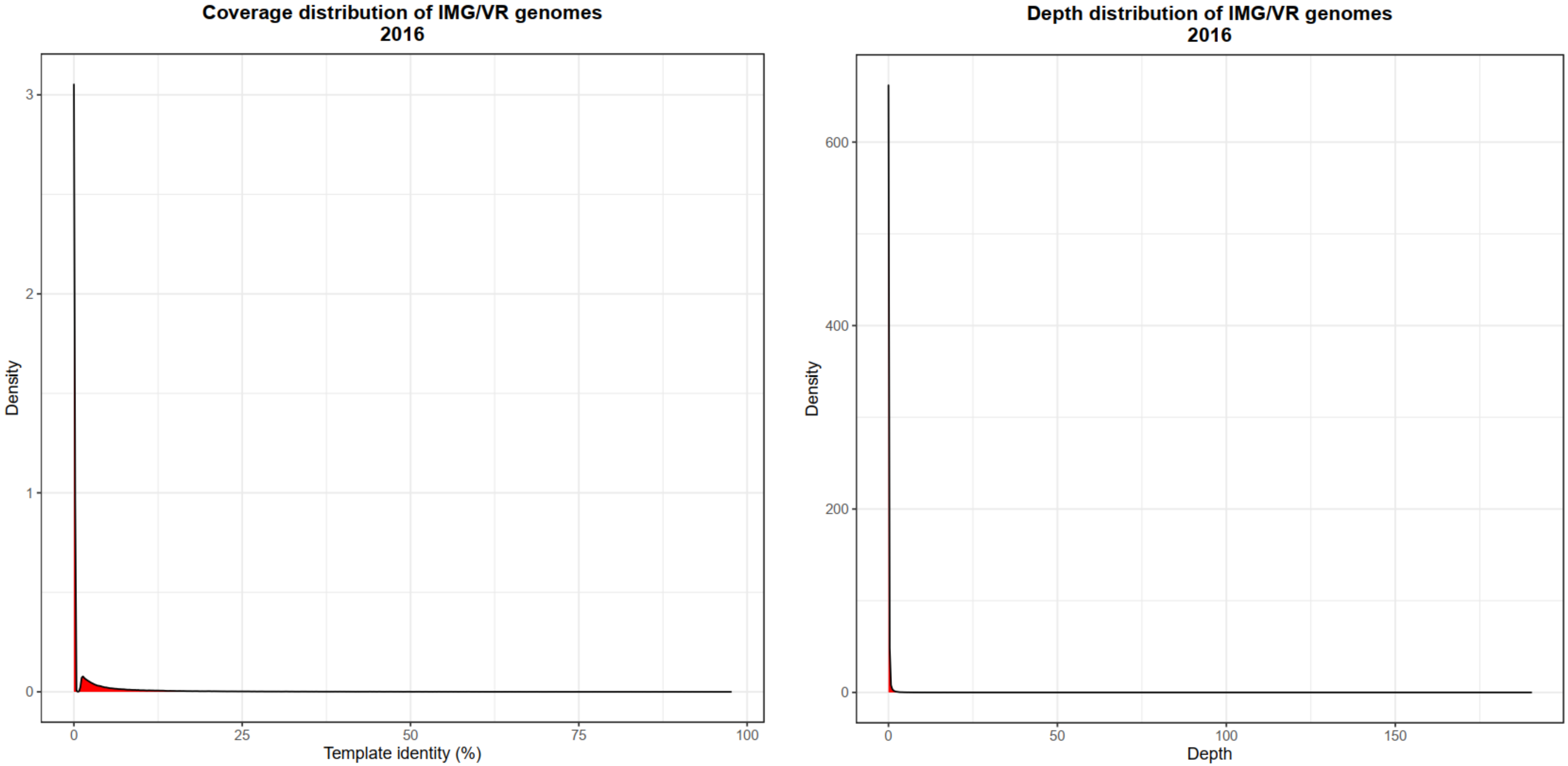
Quantile breaks of NCBI bacterial genera

Database	**Quantile breaks**	**Colour representation**[†]
NCBI bacterial genera	20	5%

[†] Percentage of datapoints in the distribution represented by a single colour, e.g. top 5% of datapoints are represented by pale yellow.



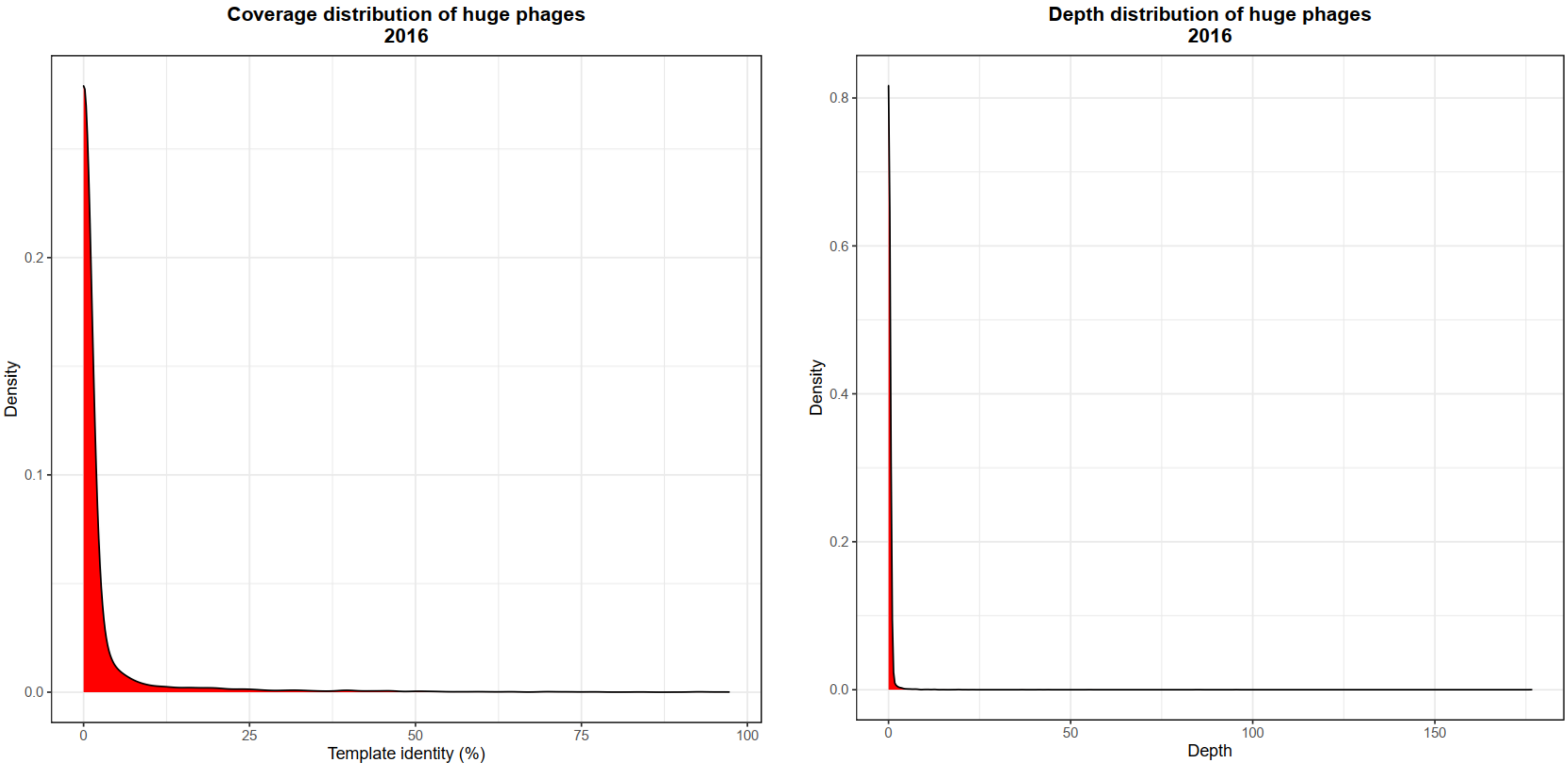
Supplementary Figure 1 Coverage (left) and depth (right) distributions for NCBI phages



Coverage distribution of IMG/VR genomes
2016

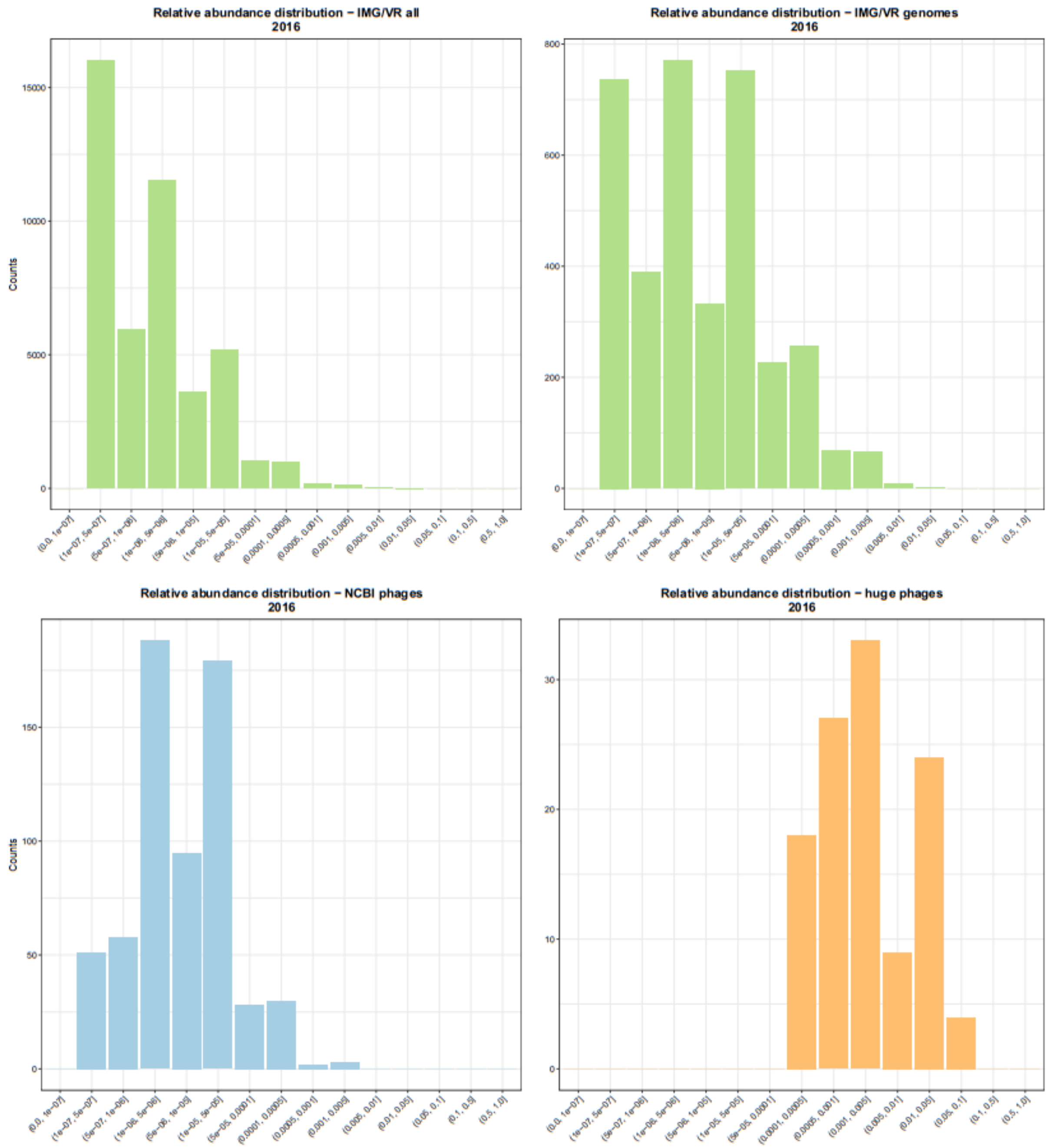
Depth distribution of IMG/VR genomes
2016

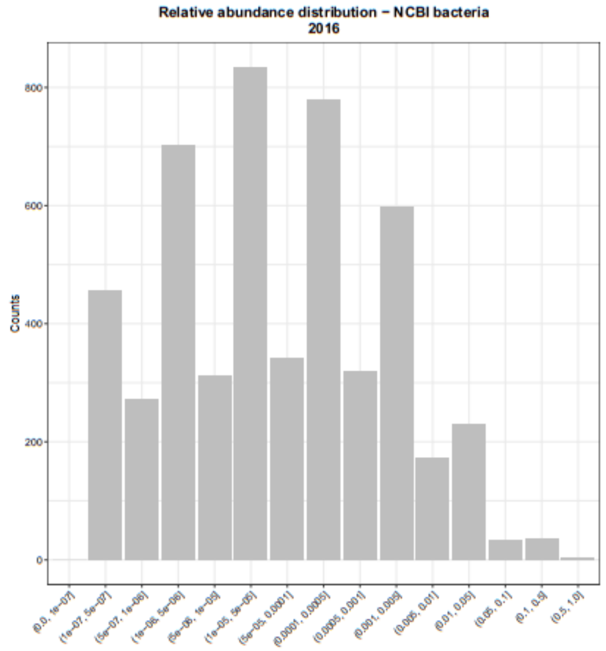
Supplementary Figure 2 Coverage (left) and depth (right) distributions for IMG/VR genomes


Coverage distribution of huge phages 2016

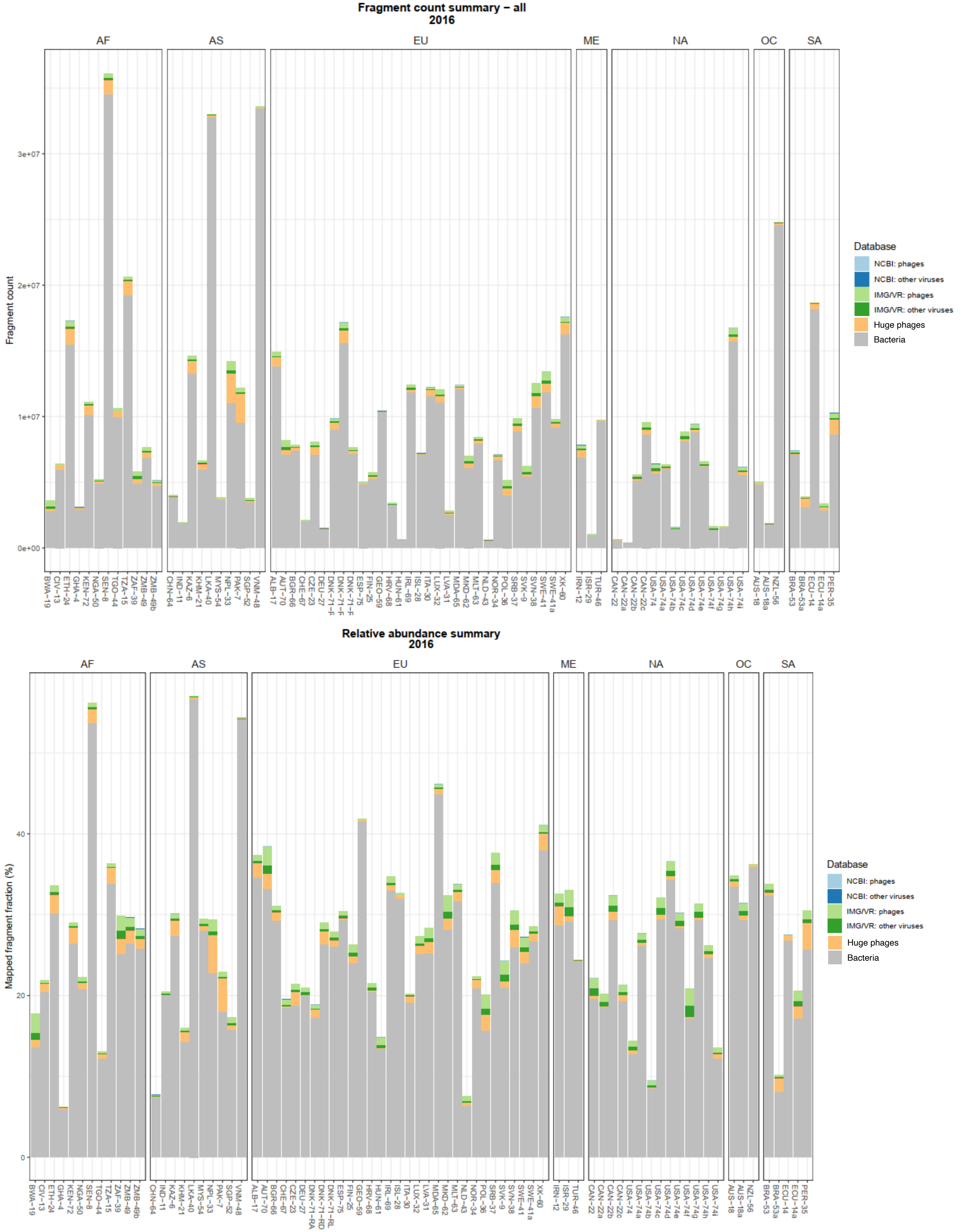
Depth distribution of huge phages 2016

Supplementary Figure 3 Coverage (left) and depth (right) distributions for huge phages.

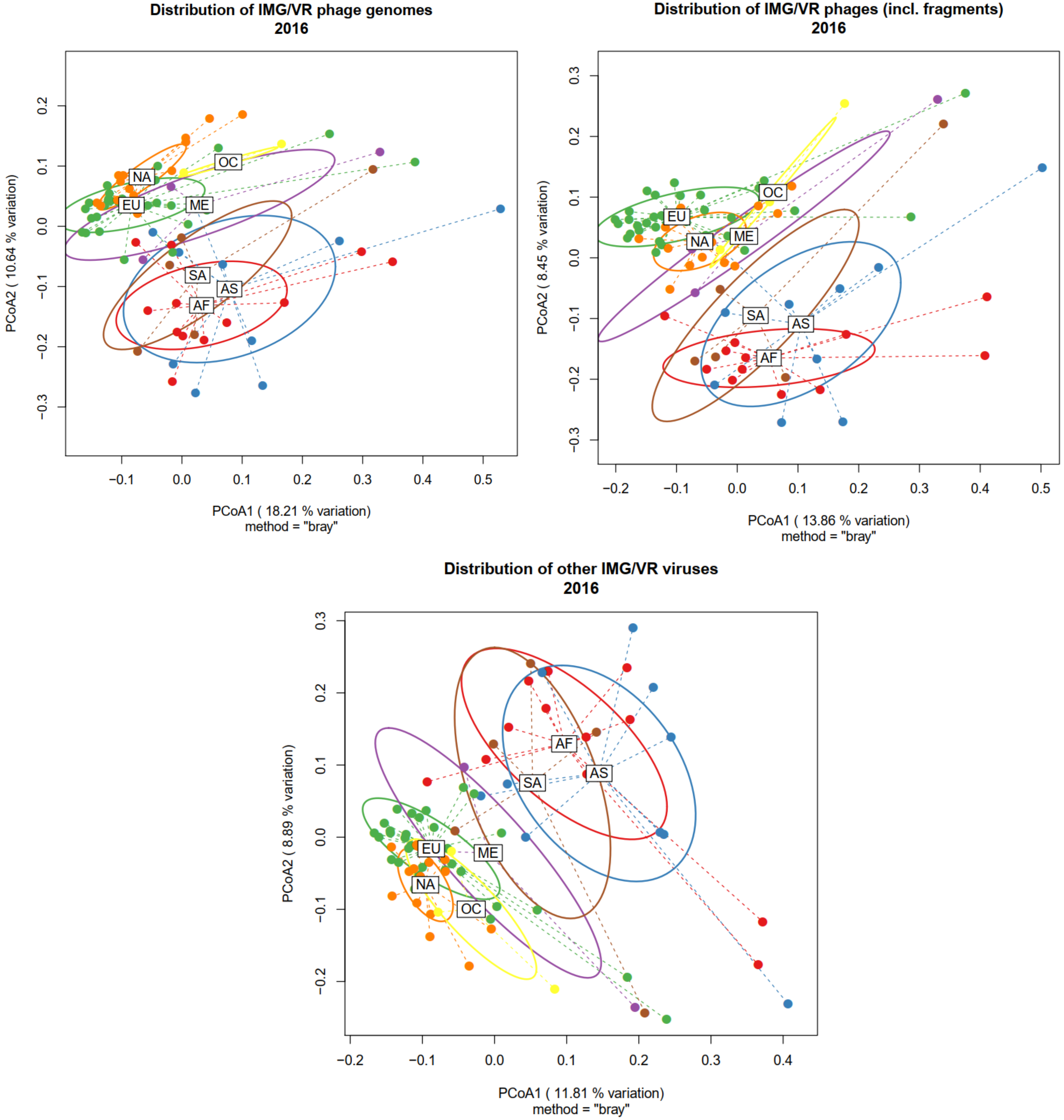




Supplementary Figure 4 Relative abundance distribution of various databases; Top left: IMG/VR all fragments; top right: IMG/VR genomes; middle left: NCBI phages; middle right: huge phages; bottom left: NCBI bacteria.

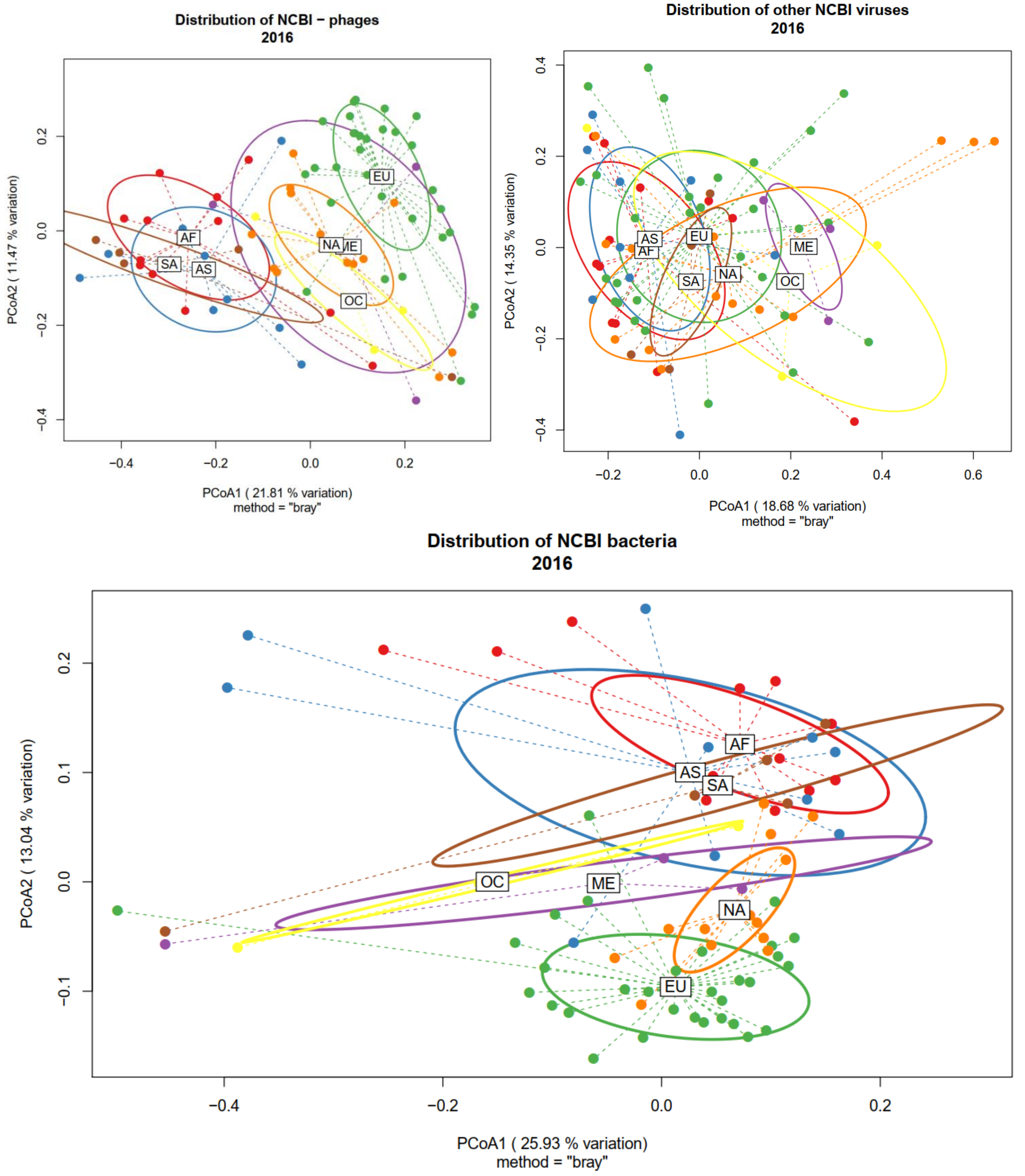


Supplementary Figure 5 Fragment mapping summary (top) and relative abundance summary (bottom) to bacterial and viral databases.
AF, Africa; AS, Asia; EU, Europe; ME, Middle East; NA, North America; OC, Oceania; SA, South America.



Supplementary Figure 6 PCoA of IMG/VR genomes (top left), all phages (incl. fragments, top right), and other viruses (bottom). Data stratified by region. Data is based on Bray-Curtis dissimilarities on Hellinger standardised relative abundances to account for phages of low-abundance. Variations are explained by X- and Y-axes (1st and 2nd coordinates).

AF, Africa; AS, Asia; EU, Europe; ME, Middle East; NA, North America; OC, Oceania; SA, South America.



Supplementary Figure 7 PCoA of NCBI phages (top left), other viruses (top right), and bacteria (bottom).

Data stratified by region. Data is based on Bray-Curtis dissimilarities of Hellinger standardised relative abundances to account for phages of low-abundance. Variations are explained by X- and Y-axes (1st and 2nd coordinates).

AF, Africa; AS, Asia; EU, Europe; ME, Middle East; NA, North America; OC, Oceania; SA, South America.

Supplementary Figure 8 Relative abundances of top 15 NCBI phages per sample.

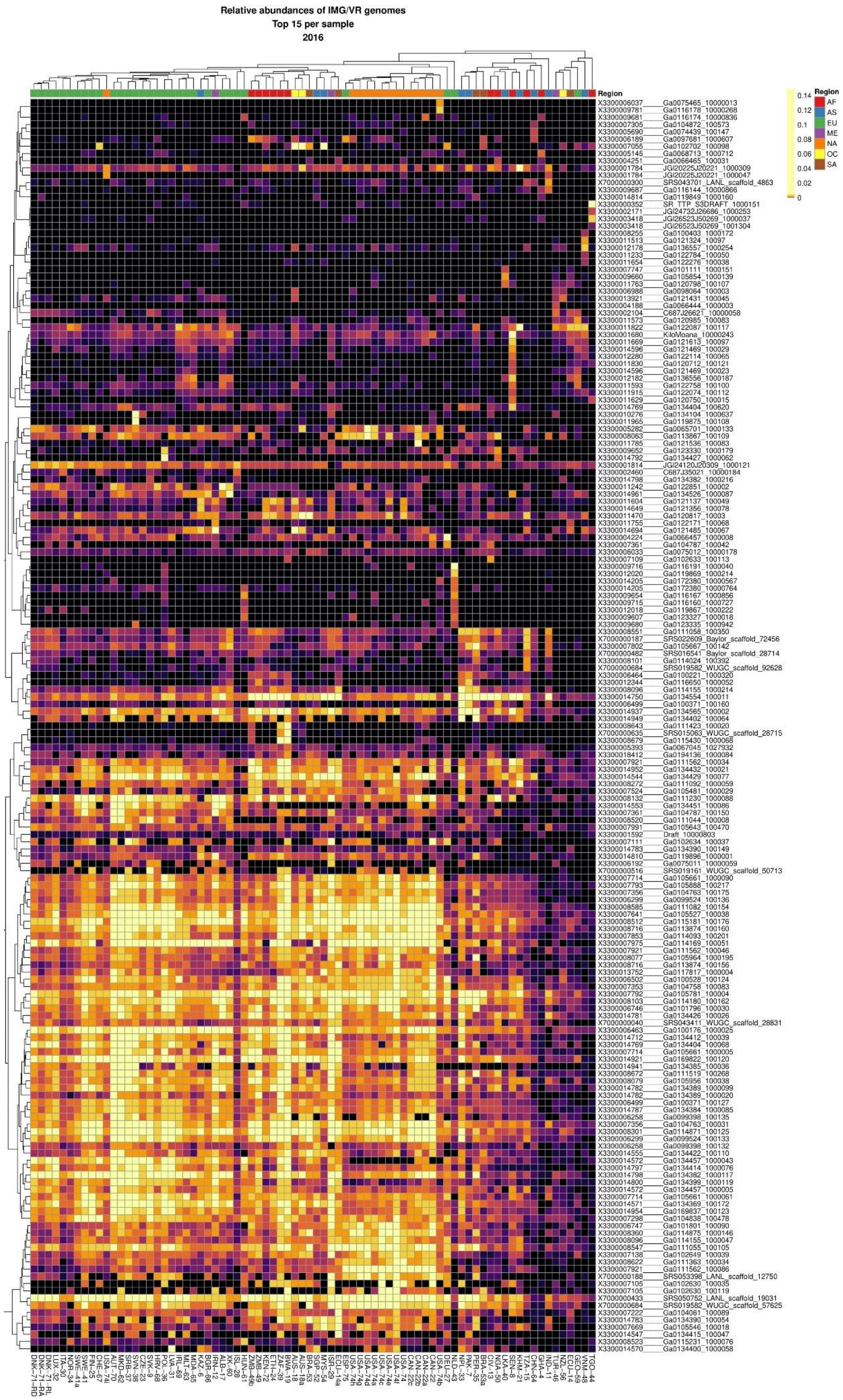
204 total phages. Colour intensity based on 0.5%-quantiles (deciles). Columns grouped by Bray-Curtis dissimilarities of Hellinger standardised relative abundances. Rows hierarchically clustered by Pearson correlation.

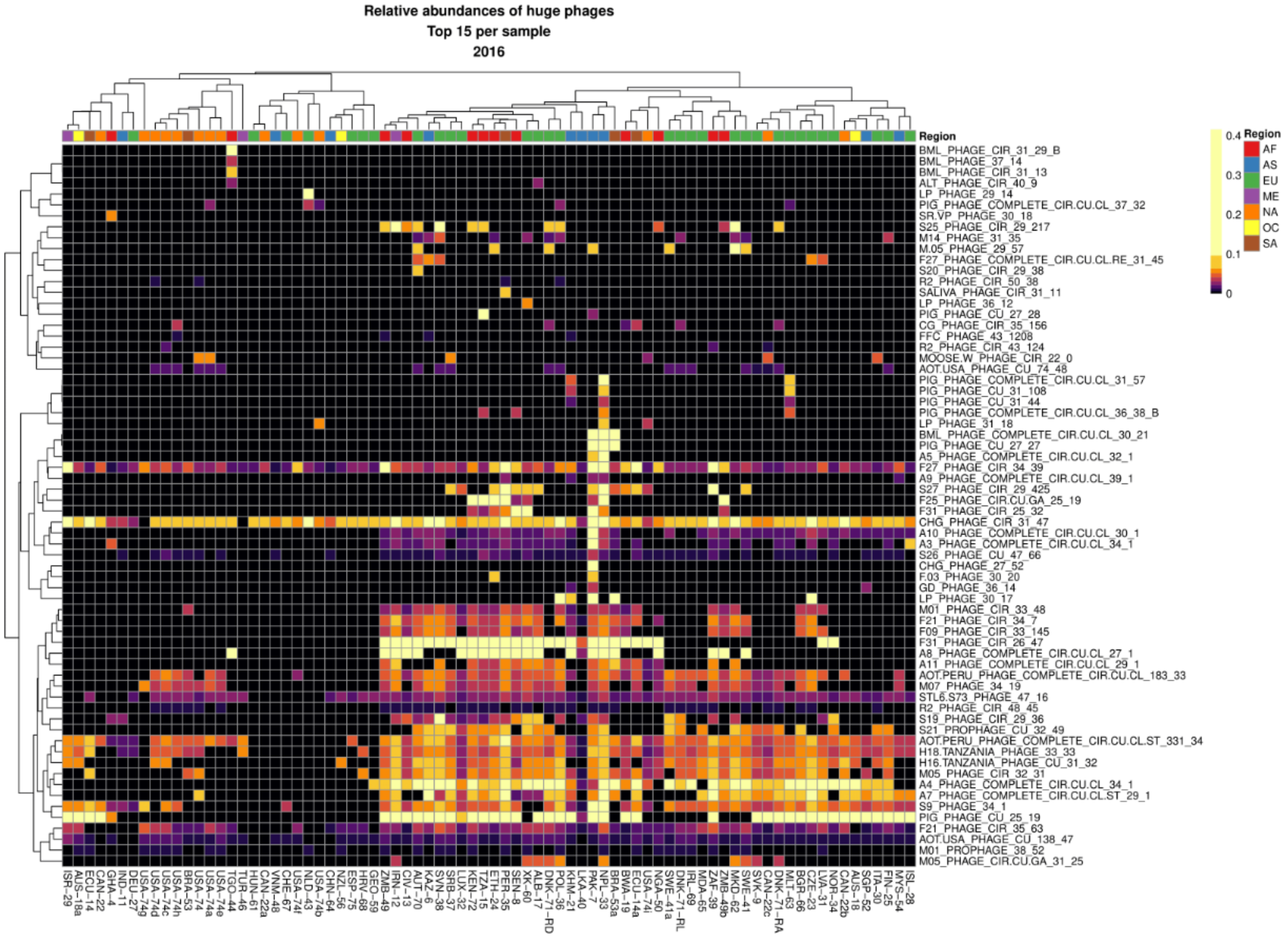
AF, Africa; AS, Asia; EU, Europe; ME, Middle East; NA, North America; OC, Oceania; SA, South America.

Supplementary Figure 9 Relative abundances of top 15 IMG/VR genomes.

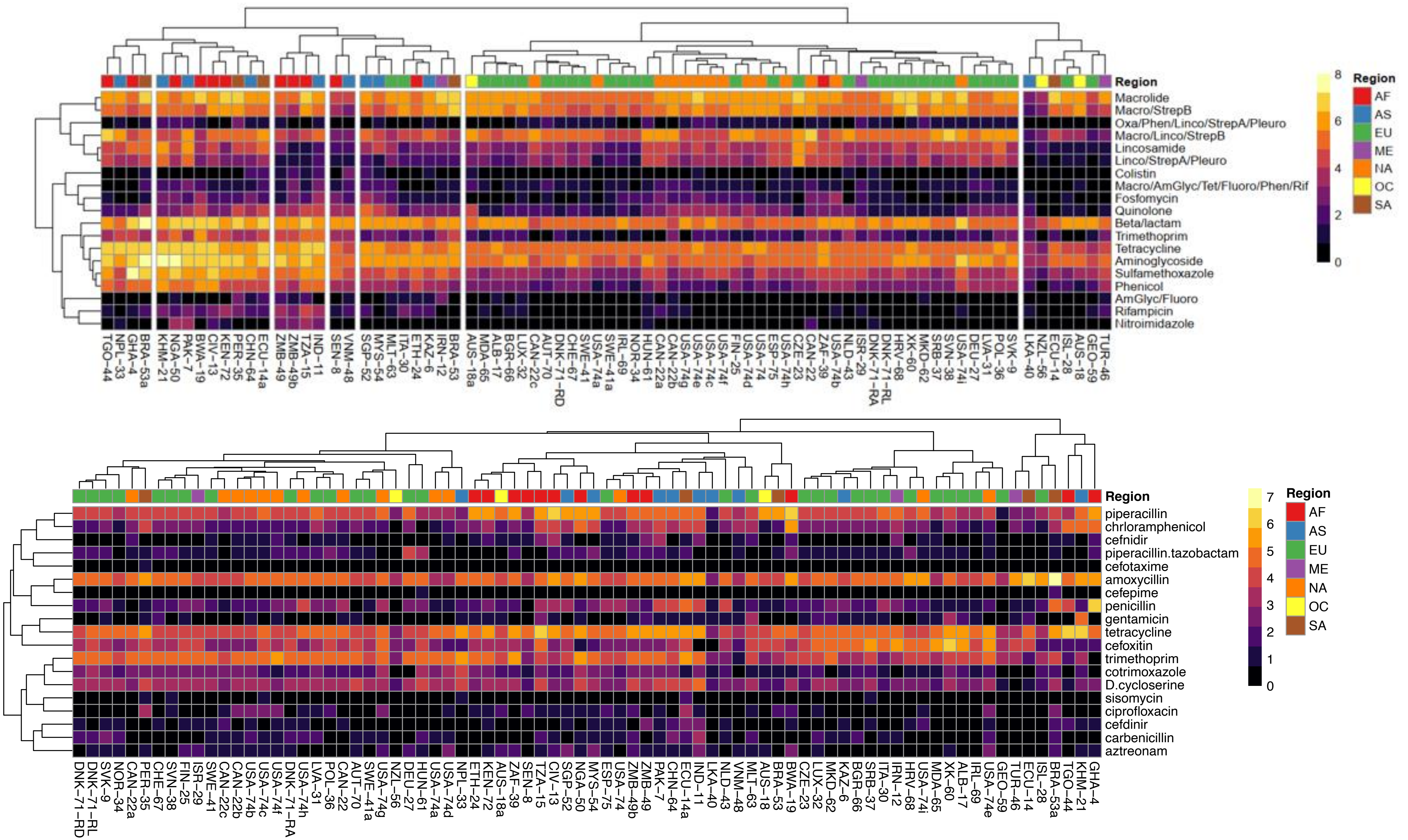
173 total phages. Colour intensity based on 6.6%-quantiles. Columns clustered according to Bray-Curtis dissimilarities of Hellinger standardised relative abundances. Row hierarchically clustered according to their Pearson correlation.

AF, Africa; AS, Asia; EU, Europe; ME, Middle East; NA, North America; OC, Oceania; SA, South America.

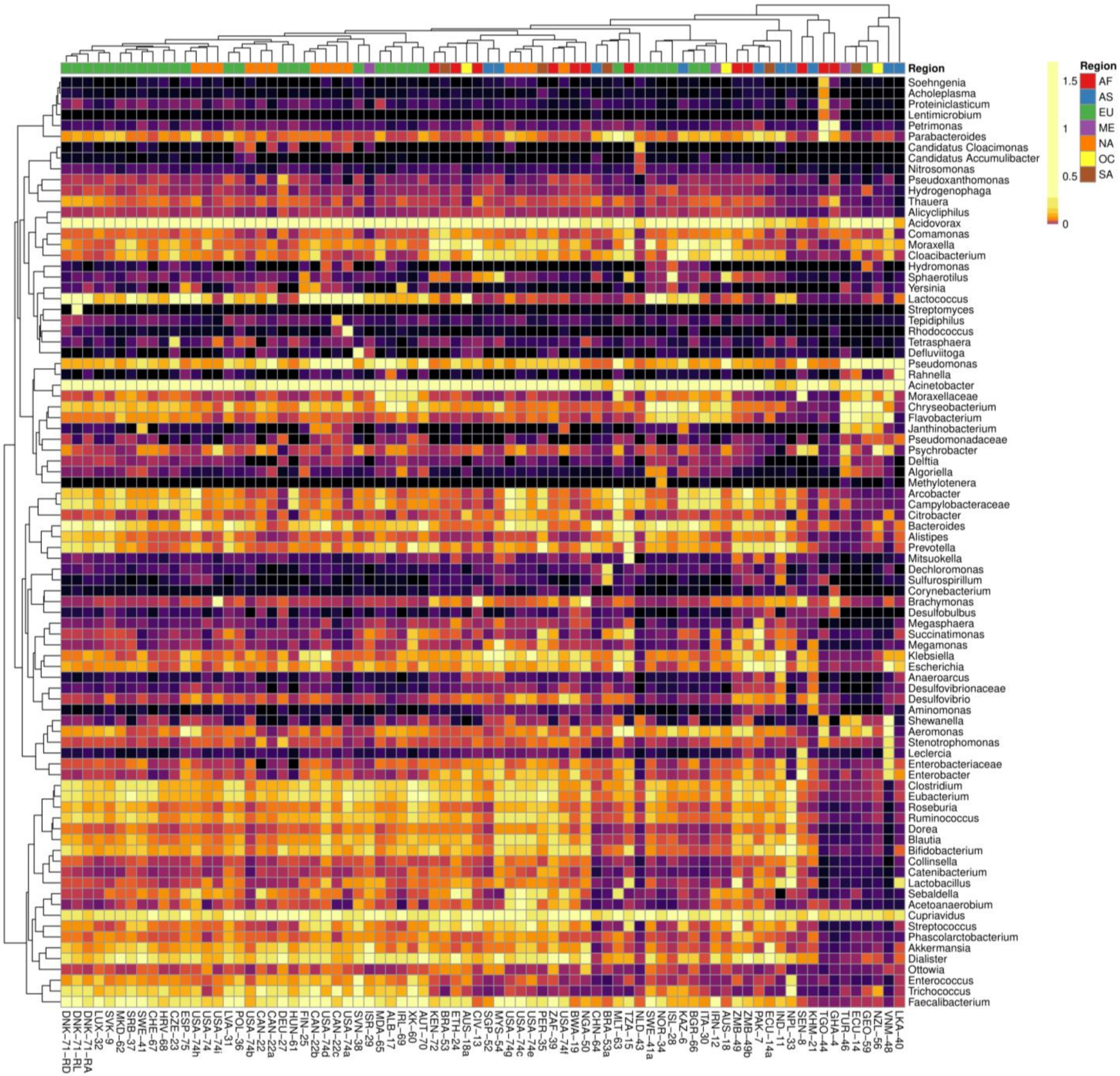




Supplementary Figure 10 Relative abundances for top 15 of huge phages per sample.
67 total phages. Colour intensity based on 3.3%-quantiles. Columns clustered according to Bray-Curtis dissimilarities of Hellinger standardised relative abundances. Rows hierarchically clustered according to Pearson correlation.
AF, Africa; AS, Asia; EU, Europe; ME, Middle East; NA, North America; OC, Oceania; SA, South America.



Supplementary Figure 11 Log-transformed FPKM of ARGs identified from ResFinder (top) and Functional Resistance (bottom). Amino, Aminoglycoside; Fluoro, Fluoroquinolone; Linco, Lincosamide; Macro, Macrolide; Oxa. Oxazolidinone; Phen, Phenicol; Pleuro, Pleuromutilin; Rif, Rifampicin; StreptoA, Streptogramin A; StreptoB, Streptogramin B; Tet, Tetracycline. AF, Africa; AS, Asia; EU, Europe; ME, Middle East; NA, North America; OC, Oceania; SA, South America



Supplementary Figure 12 Relative abundances of top 15 NCBI bacterial genera. Intensities based on 10% quantiles.
AF, Africa; AS, Asia; EU, Europe; ME, Middle East; NA, North America; OC, Oceania; SA, South America.