# Supplementary Information for:
# Lytic archaeal viruses infect abundant primary producers in Earth's crust

Janina Rahlff[1*†], Victoria Turzynski[1*], Sarah P. Esser[1], Indra Monsees[1], Till L.V. Bornemann[1], Perla Abigail Figueroa-Gonzalez[1], Frederik Schulz[2], Tanja Woyke[2], Andreas Klingl[3], Cristina Moraru[4], and Alexander J. Probst[1#]

[1]University of Duisburg-Essen, Department of Chemistry, Environmental Microbiology and Biotechnology (EMB), Group for Aquatic Microbial Ecology, Universitätsstraße 5, 45141 Essen, Germany

[2]DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA, 94598, USA

[3]Plant Development & Electron Microscopy, Biocenter LMU Munich, Großhaderner Str. 2-4, 82152 Planegg-Martinsried, Germany

[4]Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl-von-Ossietzky-University Oldenburg, PO Box 2503, Carl-von-Ossietzky-Straße 9-11, 26111, Oldenburg, Germany

*authors contributed equally

[#]Corresponding author: alexander.probst@uni-due.de

†Present address: Centre for Ecology and Evolution in Microbial Model Systems (EEMiS), Department of Biology and Environmental Science, Linnaeus University, SE-39182, Kalmar, Sweden

Supplementary Table 1: List of accession numbers for metagenomic reads, assemblies and genomes deposited in public databases. ACLF=Alpena County Library Fountain, BF= biofilm, ENA=European Nucleotide Archive, GA=Geyser Andernach, HURL=Horonobe Underground Research Laboratory, MSI=Mühlbacher Schwefelquelle Isling, SRA=Sequence Read Archive, TPA= Third Party Annotation Section of the DDBJ/ENA/GenBank databases.

Metagenomes (raw reads)

| Sample | Database | Bioproject accession | Biosample accession | Run accession |
|---|---|---|---|---|
| | | | | ERR628383 |
| MSI_2012_Biofilm | ENA | PRJEB6121 | SAMEA2779769 | ERR628383 |
| MSI_2018_Biofilm | NCBI/SRA | PRJNA628506 | SAMN14733005 | SRR11614987 |
| MSI_2018_0.1µm | NCBI/SRA | PRJNA628506 | SAMN14733006 | SRR11614986 |
| MSI_2018_post0.1µm | NCBI/SRA | PRJNA628506 | SAMN14733007 | SRR11614988 |
| HURL_250m | NCBI/SRA | PRJNA321556 | SAMN04999997 | SRR3546457 |
| HURL_140m | NCBI/SRA | PRJNA321556 | SAMN04999996 | SRR3546456 |
| GA_1_1 | NCBI/SRA | PRJNA627655 | SAMN14680028 | SRR11600163 |
| GA_1_2 | NCBI/SRA | PRJNA627655 | SAMN14680029 | SRR11600162 |
| GA_2_1 | NCBI/SRA | PRJNA627655 | SAMN14680030 | SRR11600161 |
| ACLF | NCBI/SRA | PRJNA340050 | SAMN05661201 | SRR4293692 |

Assemblies

| Sample | Database | Bioproject accession | Biosample accession | Run accession | % Rel abund. Altiarchaeota* |
|---|---|---|---|---|---|
| MSI_2012_Biofilm | NCBI/GenBank | PRJNA678866 | SAMN16815598 | JAEMOL000000000 | 90.7 |
| MSI_2018_Biofilm | NCBI/GenBank | PRJNA628506 | SAMN14733005 | JAEMOK000000000 | 97.6 |
| MSI_2018_0.1µm | NCBI/GenBank | PRJNA628506 | SAMN14733006 | JAEMOI000000000 | 38.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| MSI_2018_post0.1µm | NCBI/GenBank | PRJNA628506 | SAMN14733007 | | JAEMOJ000000000 | 74.9 |
| HURL_250m | NCBI/GenBank | PRJNA730881 | SAMN04999997 | | JAEMOG000000000 | 68.9[#] |
| HURL_140m | NCBI/GenBank | PRJNA730881 | SAMN04999996 | | JAEMOH000000000 | 77.1[#] |
| GA_1_1 | NCBI/GenBank | PRJNA627655 | SAMN14680028 | | JAEMOC000000000 | 86.1 |
| GA_1_2 | NCBI/GenBank | PRJNA627655 | SAMN14680029 | | JAEMOD000000000 | 87.1 |
| GA_2_1 | NCBI/GenBank | PRJNA627655 | SAMN14680030 | | JAEMOE000000000 | 88.1 |
| ACLF | NCBI/GenBank | PRJNA730879 | SAMN05661201 | | JAEMOF000000000 | 52.0 |

Altiarchaeota Genomes

| Sample | Database | Bioproject accession | Biosample (physical) | Biosample Genomes | WGS accession |
|---|---|---|---|---|---|
| MSI_2012_Biofilm | ENA | PRJEB6121 | SAMEA2779768 | | CCXY01000000 |
| MSI_2018_post0.1µm | NCBI/GenBank | PRJNA628506 | SAMN14733007 | SAMN18220766 | JAGTWS000000000 |
| HURL_250m | NCBI/GenBank | PRJNA726852 | SAMN04999997 | SAMN18220774 | JAGWDP000000000 |
| HURL_140m | NCBI/GenBank | PRJNA726852 | SAMN04999996 | SAMN18220775 | JAGWDQ000000000 |
| GA_1.1 | NCBI/GenBank | PRJNA627655 | SAMN14680028 | SAMN18220852 | JAGTWP000000000 |
| GA_1.2 | NCBI/GenBank | PRJNA627655 | SAMN14680029 | SAMN18220853 | JAGTWQ000000000 |
| GA_2.1 | NCBI/GenBank | PRJNA627655 | SAMN14680030 | SAMN18220854 | JAGTWR000000000 |
| ACLF | NCBI/GenBank | PRJNA726854 | SAMN05661201 | SAMN18221259 | JAGWDR000000000 |

Viral Genomes

| Sample | Database | Accession |
|---|---|---|
| Altivir_1 to Altivir_8 | Github | https://github.com/ProbstLab/viromics/tree/master/viruses/Altivir |
| Altivir_1_MSI_BF_2012 | NCBI/GenBank | MW522970.1 |
| Altivir_2_MSI_BF_2012 | NCBI/GenBank | MW522971.1 |
| Altivir_3_ACLF | TPA | BK059157 |
| Altivir_4_ACLF | TPA | BK059158 |

| | | |
|---|---|---|
| Altivir_5_ACLF | TPA | BK059161 |
| Altivir_6_ACLF | TPA | BK059159 |
| Altivir_7_ACLF | TPA | BK059160 |
| Altivir_8_HURL | TPA | BK059162 |

*Percent relative abundance compared to other community members based on rpS3 sequence analysis.
[#] since the metaSPAdes assembly of the Altiarchaeota genome was extremely fragmented and the *rpS3* gene did not assemble we used a scaffold carrying the ribosomal proteins L30 and L15 for coverage estimation of the dominant Altiarchaeum.

Supplementary Table 2: Characteristics of potential *Ca.* Altiarchaeum viruses detected in subsurface ecosystems. Viruses were clustered based on genus level and intergenomic similarity (Figure 2, Supplementary Figure 4&5). Details on functional annotation of each protein of each virus using a variety of different tools can be found in Supplementary Data 3. ACLF=Alpena County Library Fountain, BF=biofilm, HURL=Horonobe Underground Research Laboratory, MSI=Mühlbacher Schwefelquelle Isling, ORF= open reading frame, VOGDB=Virus Orthologous Groups database.

| Predicted viral clade | Sample | Coverage | Normalized coverage | Host:virus ratio | Genome length (kbps) | %GC | No. ORFs | Av. ORF length (bps) | Coding density (%) | No. of non-normalized spacer matches (associated repeat type) | Classification | Tools for identification | Functional annotations of proteins |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | |
| Altivir_1_MSI[C] | MSI_>0.1µm_2018 | 545 | 179 | 2.3 | 8.9 | 35.0 | 13 | 526 | 76.6 | 61 (1) | Putative virus | not identified | Nuclease, Geranylgeranyl transferase type i beta subunit |
| | MSI_<0.1µm_2018 | 32 | 18 | 23.4 | 8.8 | 35.2 | 13 | 514 | 75.8 | 32 (1) | | not identified | |
| | MSI_BF_2018 | 20 | 9 | 301.9 | 8.9 | 35.3 | 13 | 517 | 75.4 | 278 (1) | | not identified | |
| | MSI_BF_2012 | 4561 | 767 | 3.0 | 8.9 | 35.2 | 14 | 499 | 78.4 | 108 (1) | | VirSorter cat 3 circular | |
| Altivir_2_MSI[C] | MSI_>0.1µm_2018 | 456 | 150 | 2.8 | 17.4 | 24.0 | 35 | 421 | 84.7 | 0[P] | Putative virus | VirSorter cat 3 VOGDB | DNA methylase N-4/N-6, Transcriptional regulator, DNA Polymerase B |
| | MSI_BF_2018 | 39 | 18 | 150.2 | 22.7 | 24.7 | 43 | 455 | 86.2 | 29 (1) | Putative virus | VirSorter cat 3 VogDB | |
| | MSI_BF_2012 | 1048 | 176 | 13.1 | 20.8 | 24.6 | 39 | 450 | 84.8 | 7 (1) | Virus | VirSorter cat 3 VOGDB circular | |
| Altivir_3_ACLF | ACLF | 240 | 199 | 4.8 | 12.6 | 31.9 | 15 | 787 | 93.8 | 9 (1)<br>8 (2) | Putative virus | VirSorter cat 3 | - |
| Altivir_4_ACLF[T] | ACLF | 169 | 140 | 6.8 | 8.2 | 32.0 | 18 | 360 | 78.9 | 3 (1)<br>10 (2) | Putative virus | VirSorter cat 3 VOGDB | Capsid portal protein, Transposase |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Altivir_5_ACLF[C] | ACLF | 15 | 13 | 75.0 | 5.1 | 27.8 | 16 | 245 | 76.2 | 5 (1)<br>2 (2) | Putative virus | VirSorter cat 3<br>circular | - |
| Altivir_6_ACLF | ACLF | 13 | 11 | 85.6 | 4.6 | 35.0 | 8 | 509 | 88.8 | 18 (1)<br>9 (2) | Putative virus | VirSorter cat 3 | - |
| Altivir_7_ACLF | ACLF | 65 | 54 | 17.8 | 3.8 | 29.1 | 9 | 323 | 75.8 | 1 (1)<br>0 (2) | Putative virus | VirSorter cat 3<br>VOGDB | Major tail tube protein, DNA gyrase/topoisomerase, RNA ligase |
| Altivir_8_HURL[H,C] | HURL_250m | 1843 | 913 | 1.8 | 22.6 | 35.6 | 36 | 502 | 79.9 | 13 (1)<br>59 (2) | Virus | VirSorter cat 2<br>VOGDB<br>circular | Terminase, Structural proteins, Major capsid protein, Prohead core protein serine protease, Peptidase M3B, Minichromosome maintainance protein, PAPS reductase, Portal protein |

[C]circular genome
[H]carries hallmark genes
[T]potential transposon

[P]In this sample the virus was only recovered as a partial sequence and recruited no spacer matches from the same sample. However, the more complete genome from 2012 did have matching protospacers as shown in Figure 4.
Note: No Altiarchaeota viruses were detected in samples from HURL (140 m) and GA

Supplementary Table 3: Sampling information for biofilms (BF) collected from the Mühlbacher Schwefelquelle, Isling, Germany (MSI); FISH=fluorescence *in situ* hybridization.

| Sample | Time of sampling | Analyses | Figures |
|---|---|---|---|
| BF samples for virusFISH | 17 - 19.01.2019 | Fluorescence microscopy | Fig. 3 (A-C), Fig. 5, Supplementary Fig. 11, Supplementary Fig. 12, Supplementary Fig. 16, Supplementary Fig. 17, Supplementary Fig. 18 |
| BF samples for transmission electron microscopy | 17 - 19.10.2018 | Transmission electron microscopy | Fig. 3 (D) |
| BF, >0.1µm and <0.1µm fraction | 15 - 17.10.2018 | Metagenomics | Fig. 1, 2, 4 |
| BF | 11.2012 - 04.2012 | Metagenomics | Fig. 1, 2, 4 |

Supplementary Table 4: Assignment of viral scaffolds to assemblies. ACLF=Alpena County Library Fountain, BF=biofilm, HURL=Horonobe Underground Research Laboratory, MSI=Mühlbacher Schwefelquelle Isling.

| *Ca.* Altiarchaeum virus | Assembly Sample | Scaffold accession # | Scaffold_name (unmodified) corresponding to virus | Scaffold has been modified?* |
|---|---|---|---|---|
| Altivir_1_MSI_BF_2012 | MSI_2012_Biofilm | JAEMOL010001844.1 | IMS_noSM1map_1844_length_8923_cov_1978.89 | yes, circular scaffold was opened at position 8902 to avoid split genes[V] |
| Altivir_1_MSI_BF_2018 | MSI_2018_Biofilm | JAEMOK010000586.1 | IMS_biofilm2018_586_length_8910_cov_12.0567 | yes, reverse complement of unmodified and opened at 2094 to align with Altivir_1_MSI_BF_2012 |
| Altivir_1_MSI_>0.1µm_2018 | MSI_2018_0.1µm | JAEMOI010003611.1 | IMS_filtrate2018_3611_length_8924_cov_331.471 | yes, was opened at 4335 to align with Altivir_1_MSI_BF_2012 |
| Altivir_1_MSI_<0.1µm_2018 | MSI_2018_post0.1µm | JAEMOJ010000621.1 | IMS_vir2018_vir2018_621_length_8816_cov_19.1948 | yes, reverse complement of unmodified and opened at 2103 to align with Altivir_1_MSI_BF_2012 |
| Altivir_2_MSI_BF_2012 | MSI_2012_Biofilm | JAEMOL010000523.1 | IMS_noSM1map_523_length_20787_cov_478.723 | yes, scaffold was opened at position 820 to avoid split genes |
| Altivir_2_MSI_BF_2018 | MSI_2018_Biofilm | JAEMOK010000143.1 | IMS_biofilm2018_143_length_22687_cov_24.831 | yes, was opened at 2251 to align with Altivir_2_MSI_BF_2012 |
| Altivir_2_MSI_>0.1µm_2018 | MSI_2018_0.1µm | JAEMOI010000767.1 | IMS_filtrate2018_767_length_17396_cov_288.247 | yes, reverse complement |
| Altivir_3_ACLF | ACLF | JAEMOF010000700.1 | Sulflake_Alpenaf__700_length_12584_cov_115.502 | no |
| Altivir_4_ACLF | ACLF | JAEMOF010001180.1 | Sulflake_Alpenaf__1180_length_8205_cov_84.5962 | no |
| Altivir_5_ACLF | ACLF | JAEMOF010001897.1 | Sulflake_Alpenaf__1897_length_5148_cov_10.2435 | yes, circular scaffold was opened at position 5149 to avoid split genes |
| Altivir_6_ACLF | ACLF | JAEMOF010002130.1 | Sulflake_Alpenaf__2130_length_4588_cov_5.55703 | no |
| Altivir_7_ACLF | ACLF | JAEMOF010002504.1 | Sulflake_Alpenaf__2504_length_3832_cov_28.6254 | no |
| Altivir_8_HURL | HURL_250m | JAEMOG010001425.1 | BJP_V-250m-2014_1425_length_22641_cov_1164.08 | yes, circular scaffold was opened at position 4821 to avoid split genes[V] |

*Remark: Scaffolds had to be re-opened or changed in their orientation for alignments or due to circularity. All final scaffolds are also available at https://github.com/ProbstLab/viromics/tree/master/viruses/Altivir

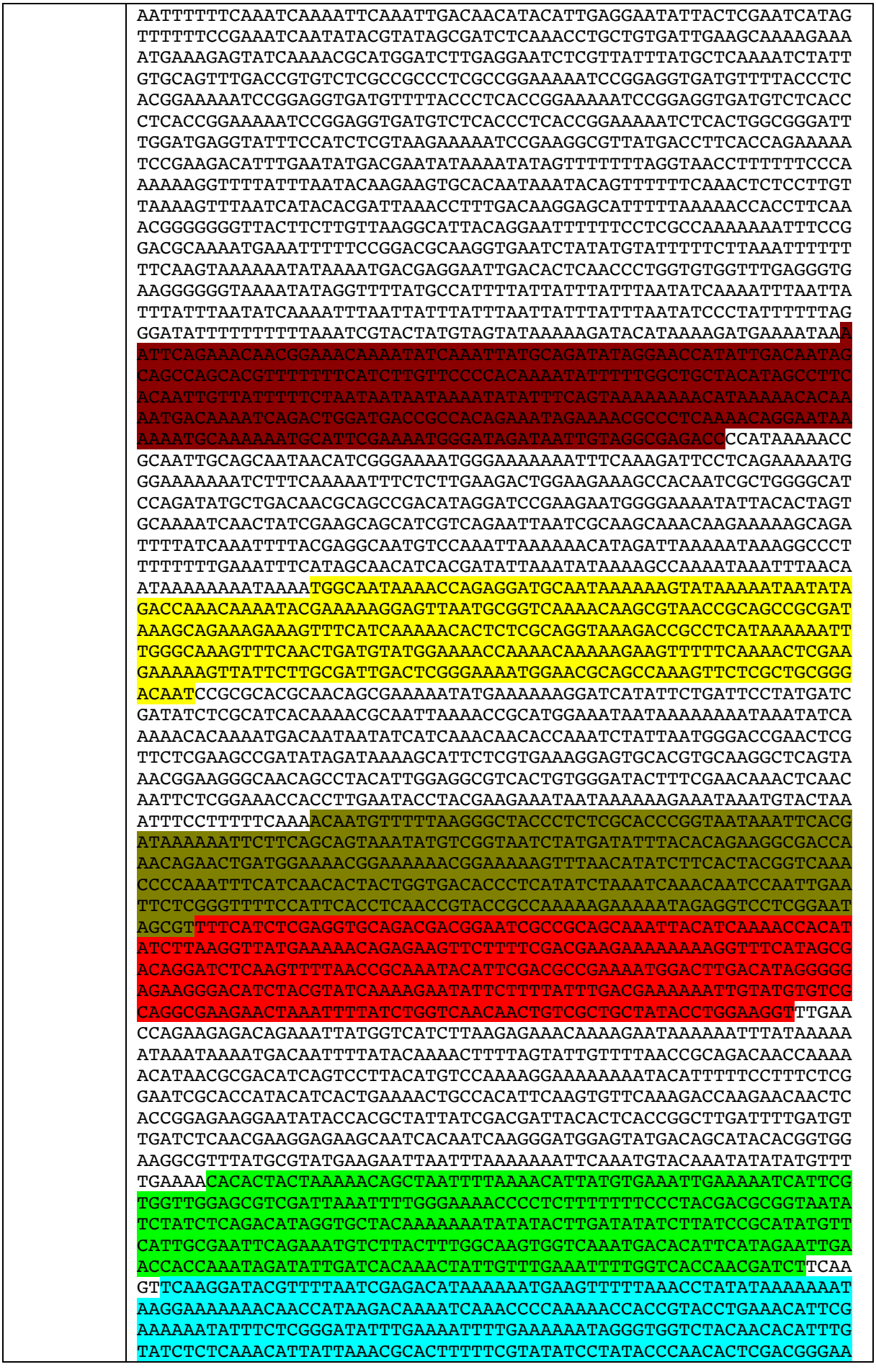
[v]Scaffold has been finally taken from VirSorter output, which produces slightly shorter scaffolds for circular viral genomes (eliminates overlaps)

Supplementary Table 5: Chemical composition of spring water from the Mühlbacher Schwefelquelle (MSI), taken from [1].

| Chemical compound | mg $L^{-1}$ |
|---|---|
| $SO_4^{2-}$ | 16 |
| $S_2O_3^{2-}$ | 14 |
| $SO_3^-$ | <1 |
| $NO_3^-$ | <1 |
| $NO_2^-$ | <0.02 |
| $NH_4^+$ | 0.33 |
| $Na^+$ | 21 |
| $Cl^-$ | 22 |
| $PO_4^{3-}$ | <0.05 |
| $Ca^{2+}$ | 79 |
| $Mg^{2+}$ | 27 |
| $K^+$ | 6.1 |
| Mn | 0.081 |
| Ni | <0.01 |
| As | <0.002 |
| Al | <0.05 |
| Sb | <0.003 |
| B | 0.13 |
| Cu | <0.005 |
| Se | <0.003 |
| Zn | <0.05 |
| Fe | 0.021 |
| $CO_2$, dissolved | 32 |
| $O_2$ | 0.13 |
| $H_2S$ | 0.5 |

Supplementary Table 6: Sequence of the circular viral genome Altivir_1_MSI_BF_2012. The regions corresponding to polynucleotide binding sites are represented in different colors.

| | |
|---|---|
| via: >VIRSorter_NODE_1844_length_8923_cov_1978_89-circular-cat_3_modified<br><br>11 polynucleotide probes (each 300 bp) from IDT (Leuven, Belgium):<br><br>Altivir_1_MSI_probe_1<br>Altivir_1_MSI_probe_2<br>Altivir_1_MSI_probe_3<br>Altivir_1_MSI_probe_4<br>Altivir_1_MSI_probe_5<br>Altivir_1_MSI_probe_6<br>Altivir_1_MSI_probe_7<br>Altivir_1_MSI_probe_8<br>Altivir_1_MSI_probe_9<br>Altivir_1_MSI_probe_10<br>Altivir_1_MSI_probe_11 | CCTGCCCAGATACTACATCTCAAACATTCCTCCAGGCATATCTTTGTGCCATACAATATCAA<br>AAAACAGGCGTAAAAGACGAACACGACTTTGAAATTTATTCCAGTTTTGACATTGAAGATCA<br>AAACGAATATGTTTATATTTCATCTGAAAGAGAAAAGAGTGACTTGTTCGAACAACAAAACG<br>CAAACTGGTATTTGATTGAAAGAGATTTACTTGGAAAAATCACGTACATCGACACAGAAACC<br>GGAGATGATTTTTCATTCAAAGAAATTGAGTCTGGGAAGCGAAAAATAGTCGAAGCGGGTGC<br>TGGAAGCATCTTCAAACTTCTCGGAAAACTAAAAACAAAATTTGGTAATTTTTTGCTCGCCA<br>ACGCAAGCCAAAAATCACTGTGGATATTAAAAAAAGAAAAATCATCAAGCTGGACAAAAAAA<br>CGGGAGCGTTCGTAATTGCCACAGGTATTTCTTCCACAACACTCATTACATCCCTCTTAAGC<br>CAAACCTCATCAACACTTCTCGCAAAAGTTCGCCTTCTCATTGAAATGCAGGGAATAGCACT<br>TTTCACGGATTTCATTGCTGAAGAAGCAGTTCAAAGTGCAGGAATGGGTGTCTTCATAGCGA<br>AAGGTCGCGACACAAAAACACTCGAACATGCTATCTCTACATACAAAAATATATATTCTCTG<br>GCTGAAAATGTACATCAATACGCACAGTGGAATATAGCGAAAAATGCCTTTGATGCCTTTTT<br>CCTCTCCGCAAAAGAAAACATAGTGATATATGAGGCACTCCTTGAAAATGCGAAAGAAACAG<br>CTGCACTTCGCGGAGAAACAGAGGACGCGGGAAAAGTCACTATATATTCTACCGAAAAATAT<br>GCAATATACCTCAACGGAATTAATACAAAAAAATACTCTCCGTCAGTCATACGAATTCCACC<br>AGGAACGCATACGATAGACCTGAAAATGAAAGACAAAATCACATATACAGAAACATTTTCAA<br>TCGAAAAATATGGCATCAAAAACATCGGGTCTGTTTCAAAAAAAATTGAACTACAAAACACA<br>ACAAAATGTCTTGATGTTGCCAACGGAACAACGATAATTCTCAACGATTTTCAACAAATCGT<br>ATTGGCAGGCGTAAAACAAAAAATTTTAACAGCAGCAGCTGAAAAAGCATCAAAAAACTGGC<br>TTCGTAAAACATGCGAAACAAGGAATATTCGCATCGAAACCGATACAGAAACTCATAACGAA<br>CATGGACAATTGTTAGGCTGGATTTTTCTTGGGGATCTAAACGTAAACGTTGAATCCGTAAA<br>ACGCGGATTTACAATAAGTAACAAAACAACAAAAAAATATGAAGATGAAATAAAAGAAGCGG<br>AAAAATTTGCCGAAAAATAATAAATTACAACAAAAAATGTTCGGAAGAGCAAATTTTTTGCC<br>AAAACACCAGAACAAAAAAGACCTACTATGGAAAGACATAGATGACTTTGTCAATTTTTATT<br>TCTTGAATCAAAAAATTTCAAAATCATACGAATTTGAACTTTACAACCCACGATGGTTTAAC<br>TCCACAAAAGCTTGGCAAATTTCATGCGCAAAGAAAATAGACCTGGTTGTAAATCGTAACGA<br>TGAAATTTATATTTTTGAAACCGACCCCCAAATTCTCGATGAAACAATCGGGCAACTTCTCA<br>CCTATCGTTTTTGGTATGAAAAGCAGGAACAAAAAAAAGTGAAAAAAATGATAGCACTATGT<br>GGGGAAATCCAAGAGCAAACTGCCGAAGTAGCACTTCTGTATGGAATAGAAGTCTGGAAAAC<br>AAATTTTCCTTCTGGATATACTCAGTATGTTTCTATGTACTAAATATGTTTCTATCTATTGA<br>ATTCATCGAAATTAAATTTTTTCTTGCCTAAATTACCCTCAAAAGTATGGGGAGCGACAAAT<br>AGAAGATTGTCCTCTTGGCCAATCTTCTAGGGATATTAAATTTTCCCCCGCCTAAATTGTTC<br>TTAAAAGTACGGGGAGCGACCGGGGGGGCTCAAACAAAAAAAAAAATGTGAATTTTGATTTTT<br>TTTTTGTTTGCTCGGAGCCTGGCGGGCTCTTGCCCGCCCGGCTCCGAGGTGGAGTCTGGTTT<br>GGGGGCAAGCGGCTTCGCCGAAAATTCTTTGGAATTTTCCCCCAAACCATCCTCCACCTCTG<br>AGCCTATCGGCTCCGAGACGCCCCCCAGCCTATCAAAACATCAGAAAAATTCTTGGGCTACG<br>GGTATGAGCCCTACGCCCAAGAATTTTTCAGAGGTTTTGATAGGCTATATATCTTCTCATTG<br>TTATAAAAATTCTCCACAAAGTCTTCCTAAATTTTCCTAAATTTTCCTAAATTTTCCTAAAT<br>TTTTCCCTCCCTTGCTCCCTCATAAGACCGCTACCATCAAACAAACTTAAGGAATACTATAT<br>ATGCCACCTCATAAGTATCTTTTTTCTGCGAAGAAAAAAGATACTTAAAAGGGCTCTGGCAT<br>ATATATCCAACCATAAGTTCGTTTGAAGGTGCGAGCTTATGAGGGGCAAGGGGGGACGAATA<br>CAAATTTTCCTAAATTTTTCCGGAAGGTGAACATCGGAGGGGAGTTAAGTCCGAATATAAAT<br>CTCCTACTTGCGAGACAAACGCCAAATTTTCGGAATGGCGAACTCGGAAAGGGGAGTTAAGC<br>TAAATTTCCCACTGCGAGACAAACGCCAAATTTTCGGAATGGCGAACTCGGAAAGGGGAGTT<br>AAGCTAAATTTCCCACTGCGAGACAAACGCCAAATTTTAAAATTTCAAAATAATAAACAAAC<br>GACCATGAAAAATATAAATCCCACAAATGTGGTGATAGGACGCAAAAAAAAATTTGAAGAAG<br>AAATGAAAGAAAAAATAGAAGAATATGTGAGGAACCATCAAGAAGAAATAGTAGAGAAAGAA<br>AAAGAGAACATGAATCCGCCTGGAACAGCAGTCGTCTGGCAAGAGTCGGGTGGGATATTTCC<br>CTCTCGCCGGAAAAATCCGAGGGCGATATGACTTGTGATTGCTTCTTCGTTGAGATTAACAA<br>GAAAACACAAACACACACAATATTTTTTATTTTTTATTTTTTAAAAGTTTTGCTCATTCCCG<br>TATTTTTAAACCTTTCACCTTTATTCAAAGGTAAATCTTTTAAATATGGTTATGTGTGCTAA<br>CTTCTGCGGTTTAAACTTTTTAACTAACACACACAAAAAAAAAGTTTATAAAAGAAAGGTAG<br>CCAAAGAAAATTATCAAAAGCAACAAAGTGGTATGTCATCGCTTAACAATTATGAAAGCGAG<br>GTTAAGTATTAATTAATTTTAAACTTTTTATCTTTATTCTTTGTTAGAAAAATTTAATTATT<br>ATTATTATTAAAAAAAATATAAAAAAAAATAAATAAAAAACAATGGAAAATATTTTTGAAAC<br>CGTGATGAAGGAACCAATAGAAATTAAAAAAAAACGCGAACAAATAGCAAGAATAAAATATC<br>TCAAAGACCGACATGGCGTCATTTCCCTATGGCGCGAAGAACCAGTCCAGATAAATCTTCGG<br>GGATATCTCCTGACTATCAGGCGTATCGAAGGTGAAACAGGAACGACAATTTCTGATGTCGC<br>GAAAGAAACGGGAAAAAACATTAAAACCGTCACGAATATTTTTAGAAATCTTGAAAAAATTG<br>AAGTGATAAAAATTTCCGAACATGCACATTTCTCTCGAAGAAAAAAAAATAAGAAAAACATA |



| | |
|---|---|
| | AAACGCAGGATTTTTCAAAGAAAAAACAGCTGGTGATATCACAACCCTCCAAACCCAGCCTG<br>GAGGAACGAAATTCTTTCTTGACAACAACCTCGTCCGTAGGACGAAAAGAATAGGTATTTCC<br>GCGTATAATATGTCAGACCTAGTAACATGCGGAGTGCATATCAGAGTCCAGGGACCATTCAT<br>CCAATCACCCATAAATGAAGTAAATCTATTCTACTTCACTGTTGCCCCGGTGACGCTCAAAA<br>CATTTGAATTTGATATTGCGTCGTTTGATGTATATCTTATTTTCGAAGCTGTTGGACCCACA<br>GGAGAATCAATAATTTTCAAAACTTATTATTTACCAATATAAAAATGGCTGATTATTTCGAA<br>TTCACCGTAAATGTCAAAGATACAGACAAAAATAATATTGAGGGTGCAAAAGTATCCATTCA<br>AGACATCACAGAAGAAACAACATCGGAAGGATATACAGACGAAAACGGAAATATTCTGTTTT<br>ACGTTCTTGCAAACAACCATATTTCCATAGAAAATGTAACATATGATCTGTATGAAATTAAA<br>TCAGGTCTCGTTTCAATACCGAAAATTACAGAAAATCTAACAGCTAAAATTGTCCTAAAAAA<br>AATAATAATAAAATATAATCTAAGTTTTACAAGTACAACTATGGAAAAAGCCTAATAAAATG<br>ACCCAAGAAAGAGCATACTGCGGGAACTGCATGAAAAAAGTAGAAATCAAAGATGGAAAAAT<br>TACAATCATTAATAAAAAAAAAGTTCTACAAGGCAAATGTCCATTTTGTGACTCCCTCTTAA<br>TAAAAAAAATATAAAAATGGCACTCGAACTTACAAAGGGTGATACCTTAAAAATAAAAATAG<br>GTGTAAAAAACGAATCTGATGGATTTACAGGAACGTACAAAATAAGTGTTGTGATCATGGAT<br>TCTAAGAATACGAAATACTACTTTAGCTACCATACTGACGGAAGCACTTTTTCAACCACTCA<br>ACAATCAATAACCGAAACACTGACGAAAGGCACAGAGTATTCTCTTTCATATTCCGCTGTCA<br>TACCTGATTACGAGGATGGCACAGTCACCGCAAATATAGGAATATACGGAGAAGAGGGGATA<br>ACAGAAATCATCGGAACAGGATGGTTTGACGCATATACTTATAGCAAAAAAAAAATGAATTT<br>GAGCTCGCTATCGGTGACCCTCGAGAAAGGCTAAC |

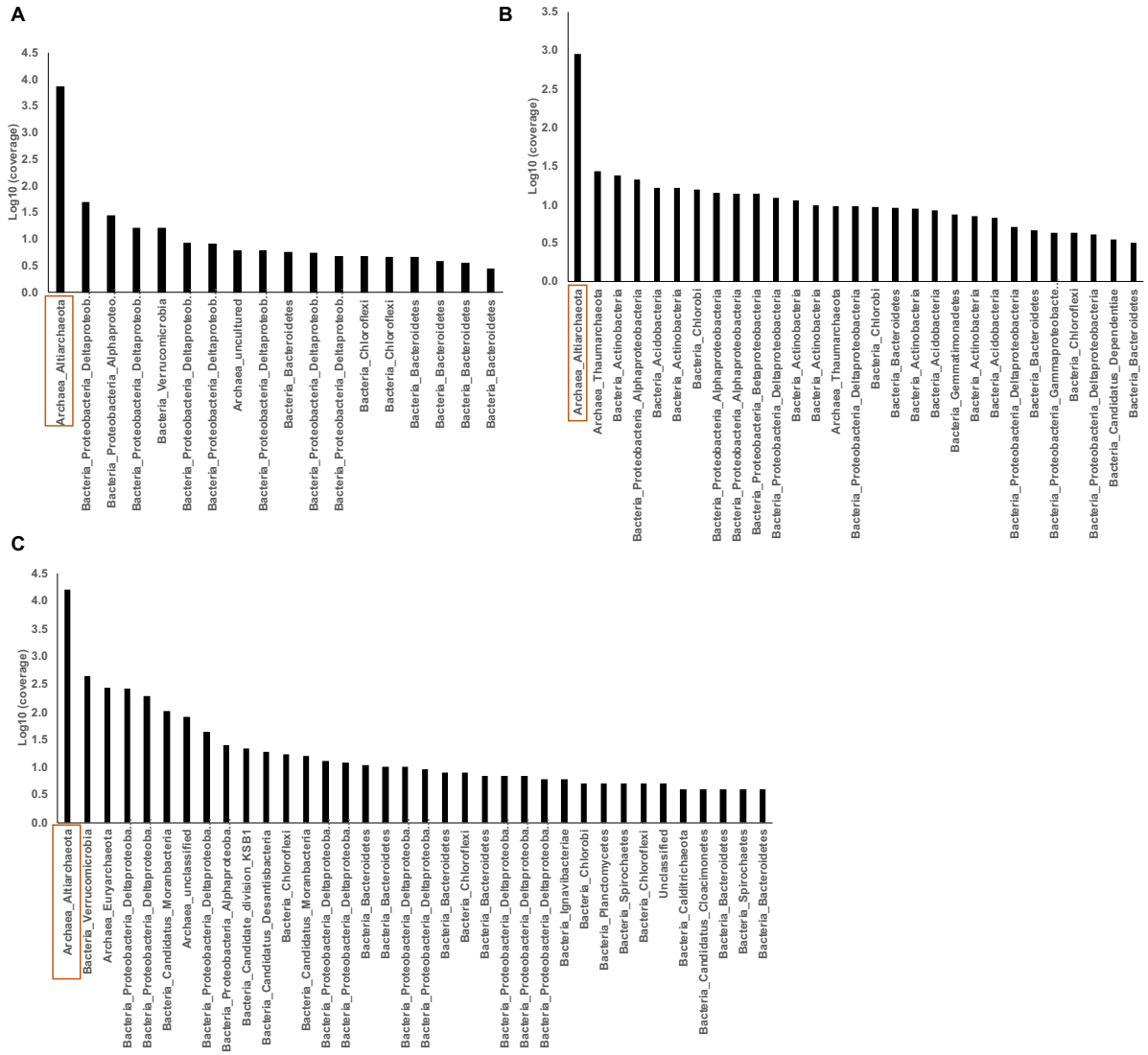
Supplementary Table 7: Sequence of the non-matching *Metallosphaera* sp. virus probe. The region colored in grey refers to the polynucleotide used.

| | |
|---|---|
| *Metallosphaera* virus (unclassified archaeal virus, 9.780 bp linear DNA), NCBI Accession no. MF443783.1<br><br>1 Polynucleotide probe (300 bp) from IDT (Leuven, Belgium):<br><br>*Metallosphaera* vir_nonprobe | CCCCCCCCCCCCATGCTGAAAGGGAGGGCGAGACCTAGCCTACCCCCCCCAGAGCAGGGG<br>GTTTAAAAGCCACGTCAGTTTGACGGATCACCACGCAAGTTGAACGCGGGGAGGGTTTAA<br>ATACCCCCCCTAGCATGAAAGCTTATTAACCCCAGGGGTTTTTGTCCTAATGCGAATGAG<br>CAAATTACAGCCCATAATACGCCCTGCCCCGCCCTGCAAGTACACTTTACGACACAAACC<br>GAAAGTAAACCTCAGAGTGAAGGGTTCTCTCCAACAGGGCAAAAAGCATTTTAACCCCCC<br>CATCACGACCTTCCGCAAAATAATTGGTCAGTCGTGGCTAAATTAAGTGAAGACTTTGTT<br>TAGGTAGTTTCGGTGGAGGGTGTGGCTAATAAGGGACAGTAAAGGTCACCATGTCGTCAA<br>ACCAGTCTAACCCAAAAAAGGAGGTCTCCCTGGAGGAGTTTTTTGAGGCCGTGAAGGACG<br>AGGTGATCCCCCTGAGAATGATAGCGGGGGATTACGTAGTCCTAGAGGGCGTCCACATGG<br>AGAACCTCAGGGACGGAAAGAACGGCAAGATAGTTGATGTGGTGGGCAGGATCGTCGTCA<br>CGAACATGGGCCTACCTGAGGGCCAGAAAGTCAGGTTCACGTTAGGCCTCTCGAACGCCA<br>TGGAAGTCGCCCATCATATAAAGGACGGCATAAAGTACACCATGATCAAGAAGGGGGAGA<br>TGAGGATAGTTGTTAATGGGTTTAGCGAGATCCCCAAGAGACTCTAGAGACGCTGAGGTA<br>TACCTAGTCAGGCCCCTGAAGGGCGTTTTTTCCCTCCAAGACCACGCTTTTCTCGTCACG<br>GTACGTGGCGTCTTCCCACGGAAGGGCGAGTTCACTCGAAAGAGCCACTGGGGCCTCATG<br>GTGGACGTGGACGTGGACGGCCTGTCCCTAAGGTTTAGGTCTAAACTCTACTTCACGTGG<br>CAAGTGGAGATGGGCGGTAGGCCGTGGGCTTTCGTGCCCGTGTACGGCTGTGCCGATATA<br>ATCCAGGAGTACGGATACCAGAGAGGTGTGGCCATATGTCAAAAATTGAAGTTGAGCAGG<br>TAGTCAAGTACATATTGGACCATCCTAGTACCTTCCTATGGTACAGGTGGTACCTGAGAC<br>AGAGGCACCACTTACCGTTCTTCTCGCTCACGTACAGTGAGGACTTACGACAGAAGGGGG<br>TGCCAGTGGACATGACCAGGGATAAGGAGGTAGTCGTGTTGGTGCCTATATACACCCCAT<br>ACGGCGTAGGCGGTGCTGAGGTCTTGTTGTCTACCCCAGATAAGGTTATGTGGAGTGACG<br>ACGACTTCGCCTTCTTCGGCAACTGGAAGTTTTACGGGAAGTTCGCGTGGATGGTCTCGC<br>TGAACCGAAAACACCCCCTGAGGCGGTACGCCCTGAACGAGATGAGGAAGGAGGCGAGGA<br>TGTATGCAGGCAAGTCCTGACATGGGGGCATTAACCCGCGCAGGGTATAGAGTGACGGCC<br>CGTTGGACGTGGAACGACGTCACTAGGGCCATGTATTGTTTTGAGACATCTAGGACAGCT<br>AACGACTTCGTGGGGTGCGTCAAAAAGCGTAGGACTGACGCCCTCGCTGTCTACCGATAC<br>CTACGAGACGTCCAGTCCCATCAGGGACTGATATATGAGGTACTCCCGCCCGCATTCCAG<br>GGCAACCCAGAGGAAGAGGAGGGGGAGGGGGAAGAGACTTGTATCACCTTCGTGGTCTAC<br>GACACGGGGTACTTCTTCCTCTGGGTAGACCCTAACCAGATCCTTCAAGACGCCTTAGAC<br>GCTAACGCGACCATGAGGATCAAGTACCCTGGGGCCTACCGTGTCATCACCACGCCGTAT<br>GACCTAGATTTCTCTCAAATAGCGTGGAGGCCCATAGTGCCGTCGGGCGAGGACTACGTG<br>ATATGTAGTAACATGGACCGTGTGGACTTCAGTGACGCGATAGACAGGTACGTCAGACAG<br>CTGAAGGCGATAATGGGGGCGATATCATGATCACGTTAGACCACGATGTACCATATGACG<br>GGTATAGACATTCGGCGAAAGGGAAACTCGTCGTTGAGTTTTGCAAGTCGTTCGAACTGA<br>GGTGTTGGTATAGGCGTTCGTCTACTGGGAACGTCCACGTGGCTATCGACGTGGACGTGG<br>ACTTCCTCAGAGAGTTGGAAATCAGGGCCGTCTTACTCGACGACCCCATGAGGATATTGA<br>ACGACTTGCGTCGGCACGCATTCCACATGCCTACTGGACGGCTCTGGGACGTGAAGGTGG<br>ATAGGGAAGGCAAAAGGCAGGCGGGCCAGTGGGAGGTCTTGTTCTCCCCTGAGTAGGGTT |

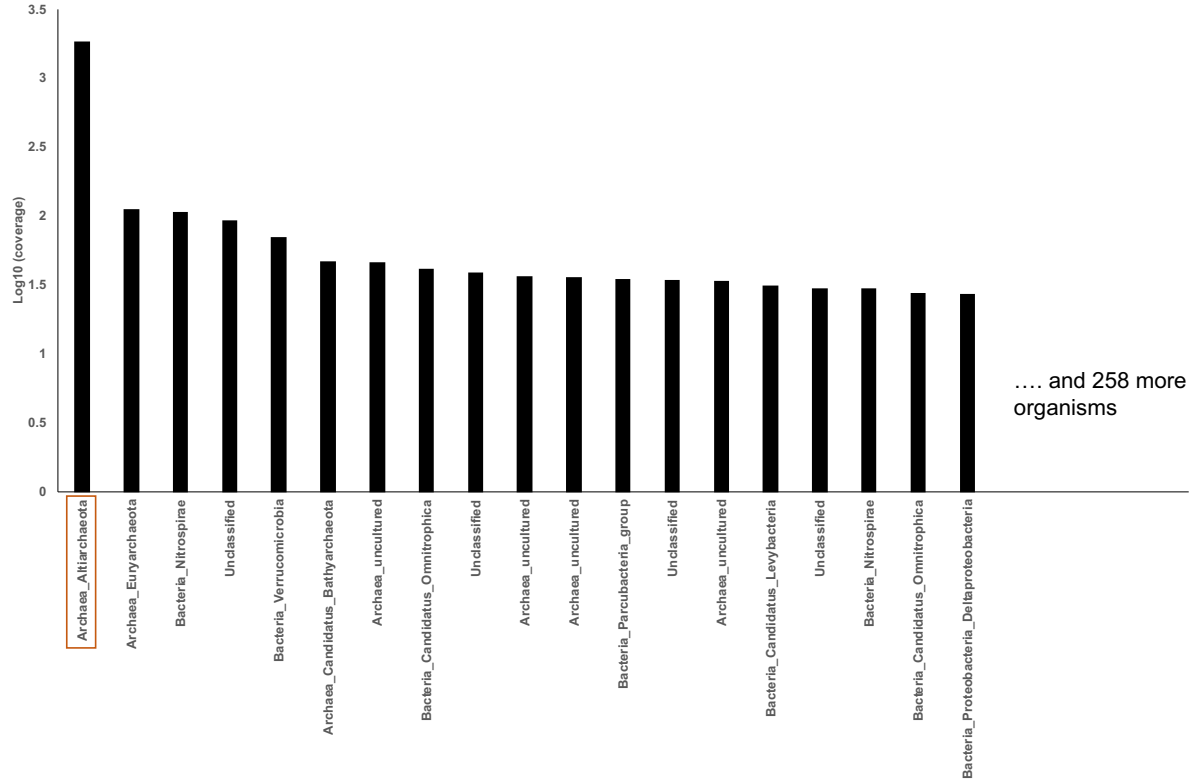
| | |
|---|---|
| | TTTTACTCCACATATCCTACTTGTATCGTATGAGTCAAAGTCAGCCTACTCAGGAACAAA<br>CCCCCCCACAAGAGGAGGGGAAGGACGAGAGCCAGTGGACGGAATGTGAAGTCTGTGGGA<br>AGAAAGTGCGGATGAACATGTACAAACGTCATATGTCTGCGGTGCATGGGTTCATTACGA<br>AAGAGGAGTACCAGAGGCTCAAAGAGGAGGTGGAGAAGACTATACAATCTACGATACACA<br>GTGCACTCCAGGAGCACTGGAAGGGGCACTACAAAAGCTTAAGCGAACTTCTTGAGGAAG<br>GGGAAAAACATGCCGAGACGTGTCCTACATGCCAAAAGGAGTTGGAGGAGTGGCTCAAGA<br>AAAAACGGAACGAGAAATACAAACCCGCTAATAGGAAGTGGCTCTAAATGACCAAGAAGA<br>AGAAGGAGGAACAGGAGGCCCCTAAGGAGGAGGGGCAGGCCACTCCCGAAACCCCAAGTC<br>CTGAAACCCCTCAGGAGCCTCAGAAGGAAGACCCCGTTCAGGTCATCATGAGGCTGACGA<br>CCGTAATACAGGAACAGCAGAAACGTATAGACCAGTTGGAAGCAAACCTCAACAGCGTGA<br>TGGAATTCCTGAAGGCACAATCCCAGGCACCCCAAGGTCAGGGAGGAGGAGGGGGTCTAG<br>TGGAGGCACTAGTACCCCTCTTAGGGAAGTTGATGGAGCCTTCGAAAGACCCCCTACGGG<br>ATATCGCCCTAGAACTAATGATCGACAGCATGAAATCGAACGTTGAGCTCTCGAAGGCCA<br>TAACACAGAAGATCATCCAAGGGGTGCAAGACCGAGTCGCGAGGAACGTCGTGGAGACTG<br>TCGCCACGGGGGTGATTACACACGAATGAGCGTATTAGAATTATCAACTTAACAAGGAAG<br>TTAATGATTGAACTGGGGGTGGGTACGTACTACCCCATGGTACAATACCGTCTTACCAGT<br>CTCCCAGAGGAACAGATAAAGAGGGCGTATAAAATCATAAGGGATGAGGTGAAAACGTGG<br>CCATAGTCCATGAGGAGGTAACGTTTCATACTGAAGTGGATGATGAGAGCGTAGAGGCAA<br>TCAGGAGGATCATAAGGGACCAGTGTGGAGAAAACCCAAAGCCTGAATGTTGGGGCCCGT<br>TGGTGTGGTCCATGTTCGACAGCGTGGCCCGTGCCATCCCGTGCCCAAAGTGCCGAGGGG<br>AGGCACTGGAATTCCTGACCTTTTTGCATGATTTAATTAACATACAGAAGGGTAGTGCTA<br>TATACAATACCCCGCAATTCCTTAGGAAGAAAAAGGAAGAGATACTGAGCCTACTCAGGA<br>CCCGAGGGATATAATATAAATAGGAGAACTGATGTAACTATGGTAGAGAGCCATGGTGTC<br>CAATAGAGAACTCGCCAAAAGGCTCAGCCAGTCGGCCAGGGAGGCGTGGAATTCAGAGGA<br>GGGCATGAGGATCAGAGAGGAGCTAAGTGCAGACTCTGCCTCCTATGGGGCGTGTCTGAG<br>GAGAGTCATGGAAAGTGGAGGAGGCAGTCGTGGTTACAAGGATTGCGCGAGGAGGTCAGG<br>AATAGGTACGGCGTTTGCCAGTGTCTGGGGGTCTACAAAGGCCTATGGTAGGCCGATGGT<br>GGAAGTAGTAGCGAGGTGACCTAGGTGGACTTCTCCAGACAAACGTGGGAGGGGCCAGTG<br>GCTGTTGGCGTCGGCGGGTACGTGTGGAACAAGGTAAAGGGGAACACGCAAGTTCAGACG<br>TTCGTGAGCAAGGTAGGTGGCAAGACGAATGCAGGGCTCATCTTAGTGGCCCTAGGCGTA<br>GTCGCTAAGGCATACAAACCCGAGTCAGAAGCCATGAATATACTGGGGTACCTGTTGGCG<br>GGACTCGGTGCGGGGGCACTCGGTGACGACTTGCCCGCCACTGGGTCACAGGCGACGTTT<br>ACGAATAAAAGCCCAGAGTACCCTAGTCCAGTTAGTGGGGTGAACGTGCTGTGAAACTCT<br>ACATGAAAACGATTGAGATCCAATATTCCATCCCTGCGAGTGCACCATCCGAGTCACAGG<br>TGCCTGGAGTGTTCATAGACCTCTCCAGTGGCACTAGCCAAGGCCAACTCCAAGTCCCCG<br>TGAACCAAGAGTGGGTCATCATAGACGTCTACAACAGGGGGTCTCAGGACGTCGGCGTAG<br>ATGCCGTGGCGACGTTCACTAAGAACAGCGTCTCCAACGTCCTCACTACGGACCCAGTCT<br>CATCCCTGAACATCTCGAACCCATCCAGACCAACCTACCCGCCAATACAGATGGCCCCAG<br>GGACGATATTGACCTCCAAAGTCACTACGCTCACGTCCAACGGCACTAGTGCGGCCGTGG<br>ATAACCTATTCATAAAGGTCAAGATCATAGACTACTCGCAGTAAACACGCGTAAGACATC<br>TCCTTTTTTAGTAGTATTTCCTTCTTTATAGTATGGCATGCCAGACTATACAAGACCTAT<br>CGATAGGCCCGTACACCATAGAATCGGGTCAGGTCTGTGTGGAGGGCACTACCTCAGGGA<br>CGGCTAGCCTGACTATGAAAGTGGGGCCGTTCACGTACACCAAGACTATCCCATGGGGGG<br>CGGTAAACCCCCAACAGACGTTGGGTACGTCCTCAGGGAGGGTCTACCTTATCGGGACGA<br>ACCCCCTGTCGTCACTCCTAGTGGACGACGTCCTAACGGTGGCTGAGTATGCACAGAAAG<br>TGGCGTCGTACTTAGGGTACAGCGGTTCGTTTTCCATAGTGAACGTGGGTTTGACGAATG<br>GCTCAGACTGCAACGACTGTGTAGTTTACGTGGACTACGTCGCCTCTTCTCCTCCTCTTG<br>CGGTAGCCCTAGTGATCGCTCTCGTGATCGTTTTCGTCATAGCTATCGTATTGGGTATCT<br>ATTTCATTTCGGCCGCGATAGAGAGTTTTCAGCCCGCACCTCCCACGCCCCCTCCTCCAG<br>GGAGTCCCCCGTCCCTGTACCAACAGTACTACCAGGAGTACGCCCAGTATTTGAAGGAGA<br>AACAAACGGCCTCAATCACGGGAGGGGTATTCGGCACGTCCACGGCCCTAGTGGCGTTAG<br>GGATAGCCATTTTAGCCTTCTTGGCGTTTACTGACCATGGTGGGAAGTGATGGTGTACCA<br>GATATACGACGGGCAATACACGGTTTACCCCGTGGGAAACCCCCAGGTCACGCTCATCAG<br>GGTAGACGTAGCGGGGAGAGTGAAAATCATGATCCTCCCCACGGTGTCAGAGGCCCTTCA<br>ACTGACCCTGAACGGAAACTCAGGTTACCTGAACCAGGGAAACGTCCTCTCGGCGGGAAA<br>TTGGTACGAGTTCGAATACGTGAGTTGTGGGAAAGACATCATAAGCATAAATGGGACACA<br>GCAAGGAGTTTACCTTAGGGTGATAGTGTATTGATTAGAGGTCAGATACACAAACTCTCC<br>AAGTGGGAGTTAAGGGACTACGCTAGGACACTAGTCCCCGTCTTTGAATCCCACGAGGTG<br>GACGGCACTAGGGACATGAAAATCGTGGACGCCGTGATATCGTACGCCATGAAAGGGACG<br>AAACCCTGGCTGAGGAGGGGGTCAGCCCTCCAGGGCTTTATAGGGCCTAGGATGTCTGTC<br>CTCGAGGGTTACCTCATCTTCCCCACGTCAGGTATTGAGCCCGTGACCATCCAGTCCACT<br>ACCCTAGAGAACTCCTACCTAGTCCTAGATATCGTGGGGGCAGGTGGAGGAGGTGGTGGT<br>GGAGGTGGTGGGAACACCACGGCCACTACCCCGTTCGCCCAGGGTGGTGGAGGTGGGGGA<br>GACGGTGGCCGTCTCATTATCAGGACCCAGGGGCCACTAGTCTCTGGGACCCAGTTCACA |

| | |
|---|---|
| | GCCCAACTGGGAGGGCCAGGTGGGACAGGTGGAGGGACAGGTGCCACAGGGACACCAGGT<br>TCAGCACCAAACGGGGCGACTCAGGTAGTCCTAGGGAACGGCACACTCACGCTCAGTGTG<br>TCAGGTGCGTCAGCCCCTACCATGCCCCCGACAGCAGGAGGACAGGAGGCAGGAGGGTCA<br>GGAGGTGCAGGAGGGTCAGCAGTGTTCACGTACTTGCCAACGACGCCCATATTCTCGGCC<br>ATACAGTTGAACCCAAGGACGTCCTTCCTCTCGAATTACTCGGGCGTACTGGACCAGGGT<br>ATTGGTGGAGACGGCGTTGGGTCTGAGTACTTCACCCTGTCCAACGCCACGCCTATATTC<br>GTCTCGACGCCACAAGGAACGAGGGTCACTGGGGGAAGCCCAGGGGGCACAGGCACCTCA<br>GCCACGGCGAACGTCCCCTCTGGGGCAATCCCGCTCAGGGGGTCAGGTGCAGGAGGAGGA<br>GGGGGACAGAACTCCTCTTCCGCCCCAACAGCGGGAGGGAACGGCGGGTCTGGAGGGCCT<br>GGACCTATCATTATCTTGGTGATAGGATGACAGACTTGGTCACCGTGGCAGTGTTAGGGG<br>TACAGACGGCCGTTTTCATCTACGCTAGTGCCAAACTGAGGAAGATGAGCGAGATGGCCG<br>ACAAGTTGGCGTCAGCCCTGTCCAACATAGCAAAGGAGATAGAGACGGCGTCCATGGACA<br>TGAAAAGCATACAAAGGGAGATGACAGAACTGGAAGACTTAATTCGGACAGCACTGAGGG<br>AATAGGTATGAGCTGTCAGACATGTGGTCAGCAGGGCGTGTGTTACGTCCTGGAACACGT<br>GGGTACTGAGCCCATCTATATAAACGGGCAACAGTACGGCCAGGGCGACTTTTGTTTCTC<br>CTCCCCTGAGCTCACGATAGAATACCAGGGCGTACAGTACCAGATCGACATGTCCTACGC<br>CCCCACAGTGCAGAACATATACGGGTGTTGTCCCTCAGGTACCACAGCCAAACTCACGAC<br>GGTGACTACGATACAGGGACAGAACACTCCCATATTCGTCCCGTATGCCGTCCTAGTGTC<br>TCAAGGTACCACGTCCACTAGTAGTACCACGCCTAGTGTCCTAAGTAAGGTACCGTGGTG<br>GCTCATCATACTACTCGTCATACTGGGGGCTATACTCCTAGGAGGAGGAAACCCATGAAT<br>TACACTTGGTACGTGCAGAACCAAGACCCCTCGACGATCCAGTTCTGTGTCATGCAGTCT<br>GGAGGGTATAACAACTGCACCTCAATACCAGGGAACAGCACAGAGCCGTTCACGATACCA<br>GACATGGACGTGCAGATATATGGCACCCCCACGGGGTACTACGTGTACTACTCTAGCGTT<br>GGGGTGAAGACTAGTACCAAGTCCCTCCCGTCATCAGGATCAACGATCACGGTGCCCCCA<br>TACACGGGATGTGACCCCCTACTCACGCAAGGACAGGGGTACCCCCCACTCGTGCCGTGT<br>TCGTCCCCGTTGATCTTCCTGAAGAGCGAGAGCACCCCTGCCATGTTTATCGAGAACTGG<br>GGGGCAGAGACGTCAGGGTTTTCATACGGGTACGGGATAGCGGGGAGCCAATTGGCCATA<br>GCGACAGGGCCAAACTGGGTATACTACCGACAGTACCCCCAGGCACAGGTATACCTTATC<br>GGAAACTGGAAGGTGGTGAACGGAAACTACGTAGACATGGACTCGGGGAAAACGTTCTGT<br>GGGTGCCAGGGGAAGAGCGTGTACGTCATACCCACGTCTGACCTACCTGGGCCGTCCTCA<br>GGGACTACGTCCACAGGGACTAGTACCACGGGGACTTCCACGAGTACAACAGGGACTCCC<br>ACAGGGACGTCCACTCAGCAGACGACACAGCCCACGACAGGTATCCTGAACGTGGCTAAC<br>CAGTGTTCGACGGGGGTAGTCGTGTACGCGGGAGGGAAAACGGCATACGCACCCCCATAC<br>CATTCCATCAGCCTAGAAGTACCCATCCCCACGGGGTATACGCTTATTAGCCAGGGTCAG<br>ACAGTTGGTAAGGGGACGGTGTACCAGAGCGGGGAGTACGTGTCCCCAAGTTCTTGTCCC<br>TCTGTTAGCCCTACACCCAGCCCTACCCCGACACCGTCCCCAACTGGGGGACAGACCACT<br>TCCACGACTACGACTACCACGACTACCCAGACCACTTCAACACAGACTACCCAGAACACA<br>CAGCCCCCAACGACGCCTAGCATGTCGTGGGGTCTTATCCTACTCTTGGTAATCCTGGGG<br>GCACTAGCCCTCTCAAGTTAAGCTTTTTTAGTCCAGGTCTCCCATTATACCTGAGCGGTG<br>CGAGGTGGATGTGACGGGAGGCTCTGGGAATGTCCGAGAGACGGAGGCGTACCCGTCCTC<br>CACTGCACCGCACTAGGTGAGTCACATGAGCCAAGTCCCAAAGAAGAGACTCGGGGCAAG<br>GGTCATCAGAGCGGTTTTCAACGGGGTCTCCTTAGGCATCCTAGGAGGGGCAGGAGTGTA<br>CGTCCTAGCCCAGGGGGTGAACACGTTATCAGGCACCACTACTTTGAACCCTACGGCGTT<br>TCTCCTGTTGGTCTTGGGGGCGTCCATCACGGCCAGTGTGGGGATAGAACTCTCCAAGGA<br>CCTAAGCGGTGAGTAGTCTCAGTCAAGTCTCCTTTATTTTACCCCCTGAACCAATAGACC<br>CAAAATGGATGTCCAAACCCAACTCAAAGAATGGATTTCCCTGTTGGACGACAGGCTGTC<br>GTCCATAGAAATGAGCCTCAAGTCCCTCCAAAAGAGACTTGAGCAGGCAGAAAAGCGACA<br>AGAAGAACTTGAGGAGGCGTTCGACCAGCTGAAAGAAGAAATAGACGACTGTGTGGCTGA<br>CACGACCTACCTCCACGACGTACTGGAGGAGGTCCAAGACGACATAGACGACCTAAGGAA<br>GTGAGCCCCGTGGAGTTCGTATACAAGATCAACGACGACACGGTCACAGTGTGGGTCACT<br>GACGAGAAGACGGGGAAGAAAGTGGCGTCCCTACAAATGCCGAAGAAGGACGTGGACTAC<br>GTCGCGAGACGTGCAATACAAGAACTCGACATAGCCCCAGATGAGGCATACGACTTAGCG<br>GTCTACTTCACCTACGTTAAGGTCACCTGGGGCCTCCTCTGAACCTCTTTTTTCTCTCGT<br>ACTTACCCCTGACCCTACGGTCAGGCTCTACTTCTCTCACGAACCTGACTCCCCCATCCT<br>CATCAAACATATGATTTATTTCACAGCAGGGCTTTATATAAATTGCGGAAGGTCGTGATG<br>GGGGGGTTAAAATGCTTTTTGCCCTGTTGGAGAGAACCCTTCACTCTGAGGTTTACTTTC<br>GGTTTGTGTCGTTAAGTGTACTTGCAGGGCGGGGCAGGGCGTATTATGGGCTGTAATTTG<br>CTCATTCGCATTAGGACAAAAACCCCTGGGGTTAATAAGCTTTCATGCTAGGGGGGGTAT<br>TTAAACCCTCCCCGCGTTCAACTTGCGTGGTGATCCGTCAAACTGACGTGGCTTTTAAAC<br>CCCCTGCTCTGGGGGGGGTAGGCTAGGTCTCGCCCTCCCTTTCGGCATGGGGGGGGGGGG |

Supplementary Figure 1: Rank abundance curves for metagenomes based on ribosomal protein S3 indicating dominance of Altiarchaeota in the MSI ecosystem. for A) MSI_BF_2018, B) MSI_<0.1µm_2018, C) MSI_BF_2012, and D) MSI_>0.1µm_2018 (stretched over two panels). Assemblies for 2018 samples were performed with MetaSPADES 3.10 [2], whereas for the 2012 assembly MetaSPADES 3.11 has been used.

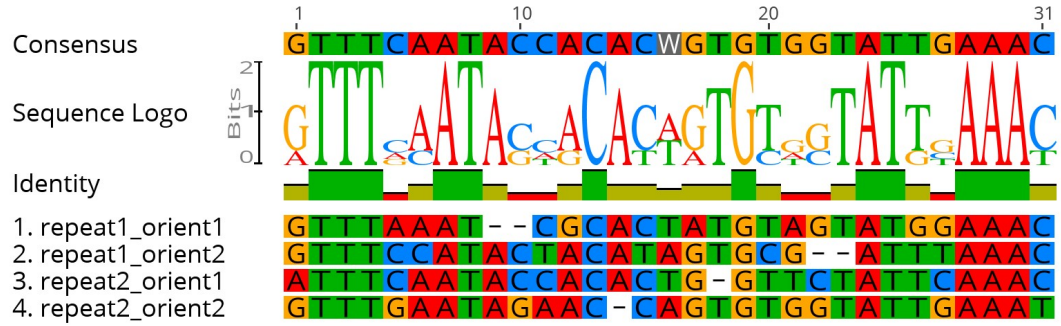


D

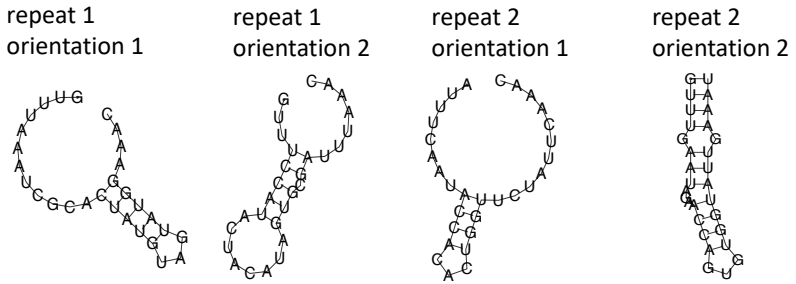

Log10 (coverage)
3.5
3
2.5
2
1.5
1
0.5
0
Archaea_Altiarchaeota
Archaea_Euryarchaeota
Bacteria_Nitrospinae
Unclassified
Bacteria_Verrucomicrobia
Archaea_Candidatus_Bathyarchaeota
Archaea_uncultured
Bacteria_Candidatus_Omnitrophica
Unclassified
Archaea_uncultured
Archaea_uncultured
Bacteria_Parcubacteria_group
Unclassified
Archaea_uncultured
Bacteria_Candidatus_Levybacteria
Unclassified
Bacteria_Nitrospinae
Bacteria_Candidatus_Omnitrophica
Bacteria_Proteobacteria_Deltaproteobacteria
…. and 258 more organisms

Supplementary Figure 2: Two distinct direct repeat sequences of CRISPR arrays derived from Altiarchaeota and formation of secondary structures. A) Alignment of CRISPR repeat sequences from Altiarchaeota genomes obtained from different habitats (Supplementary Data 1). All direct repeat sequences end with a AAA(N) motif. Alignment was performed using MUSCLE within Geneious v.11.1.5. [3]. B) CRISPR RNA (cRNA) secondary structure predictions of the different repeats and their orientations predicted by RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi) [4].

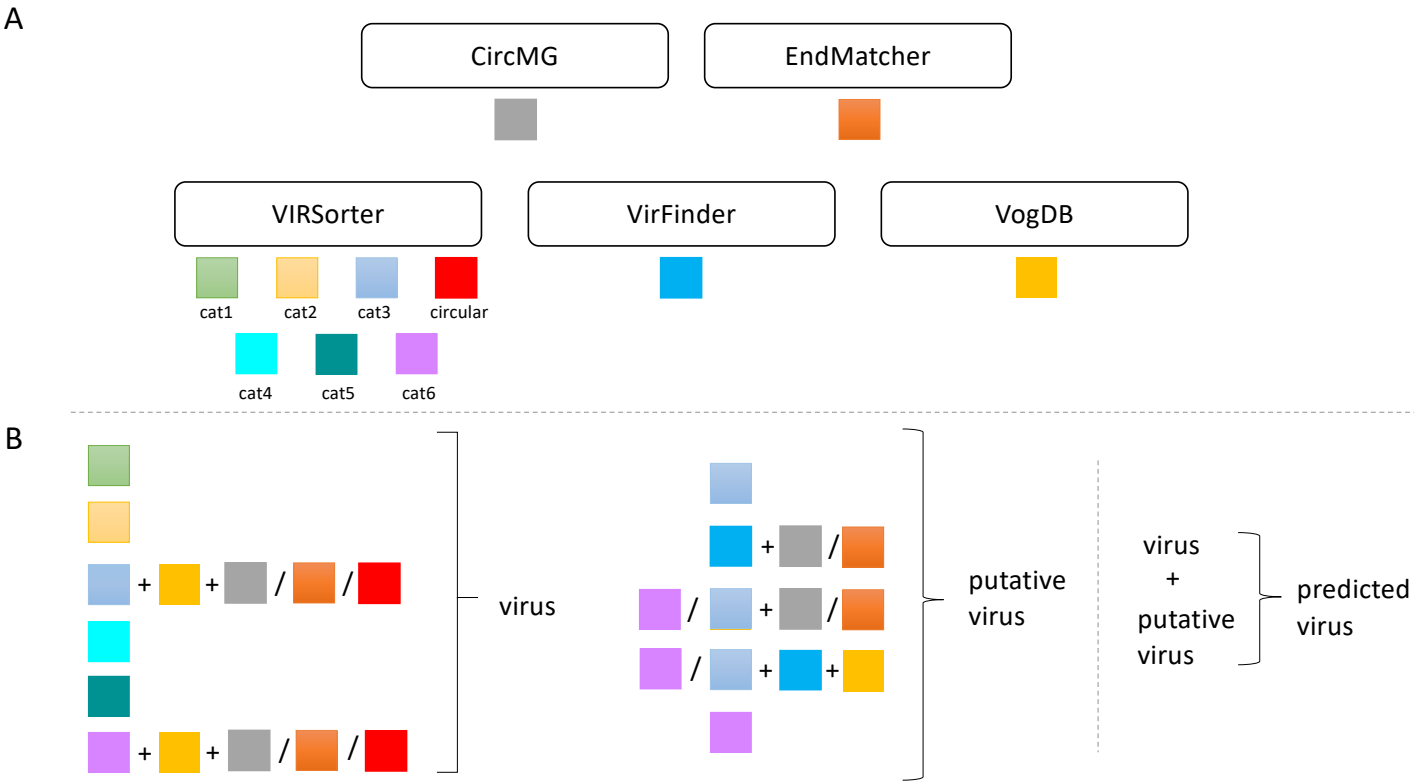
A



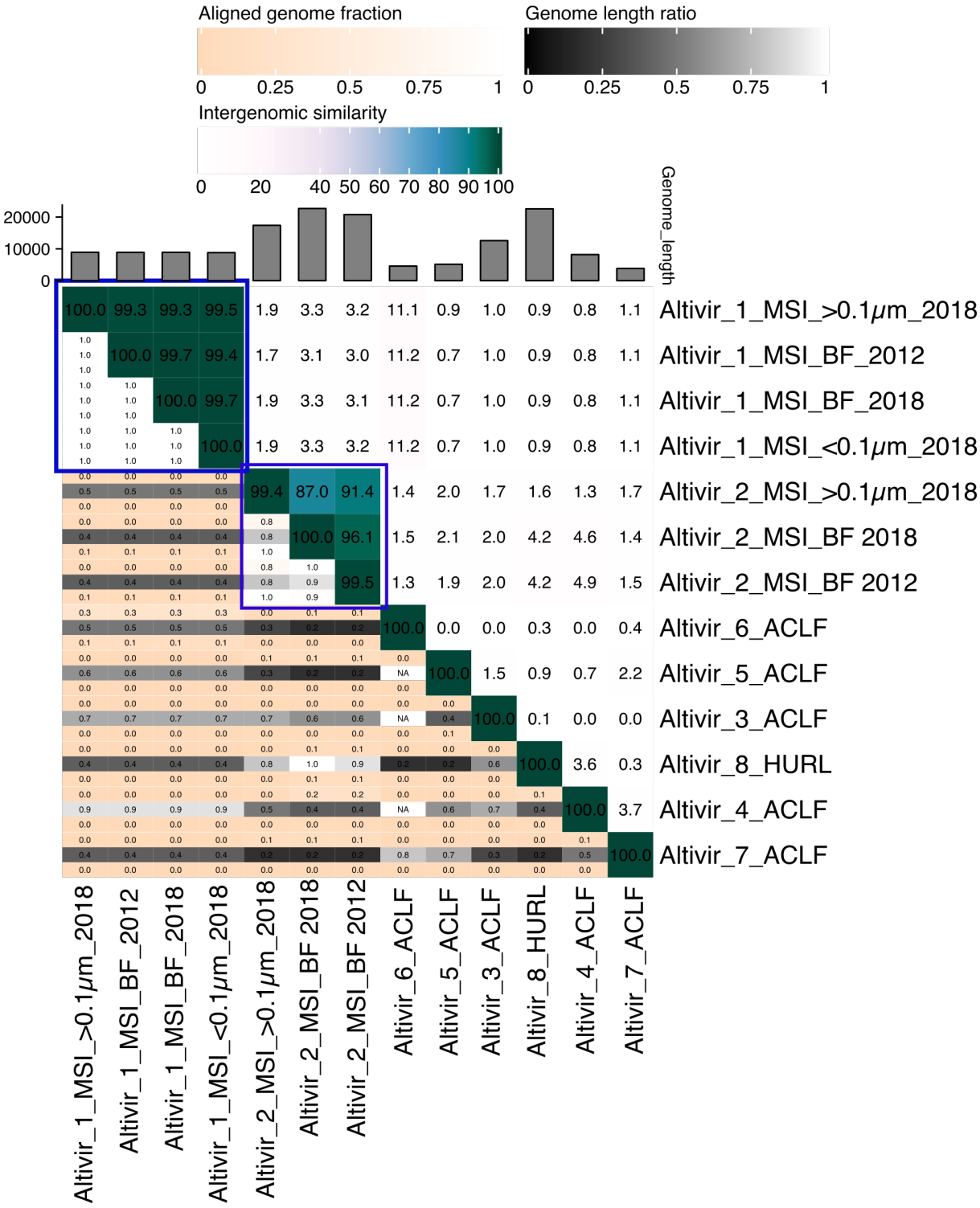

B



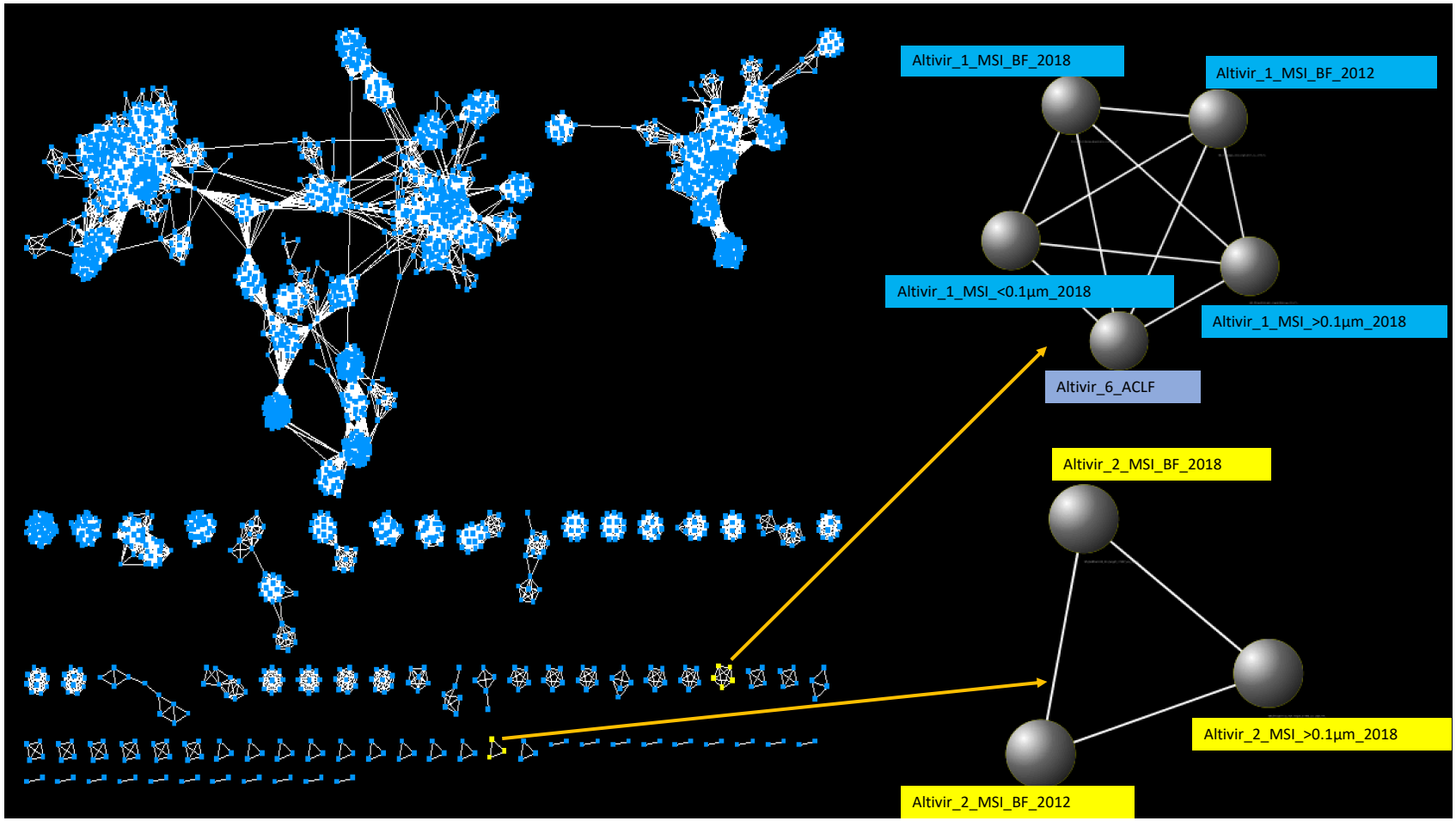
Supplementary Figure 3: Classification scheme for viral scaffolds. A) Bioinformatic tools (VirSorter v.1 [5], VirFinder v.1.1 [6], VOGDB [7] (version Vog93, e-value cut-off $10^{-5}$), CircMG [8] and EndMatcher (https://github.com/ProbstLab/viromics/tree/master/Endmatcher) used for detecting viral scaffolds; B) Combination of results and how it leads to classification of predicted viruses into putative viruses and viruses. Modified from Eßer [9]. VirFinder [6] hits were false discovery rate-corrected using the package q value [10] in the R programming environment [11] applying a p-value cut-off of <0.05. Colors indicate different tools as follows: grey=CircMG, orange=Endmatcher, blue=VirFinder, yellow=VogDB, various colors for VirSorter categories.



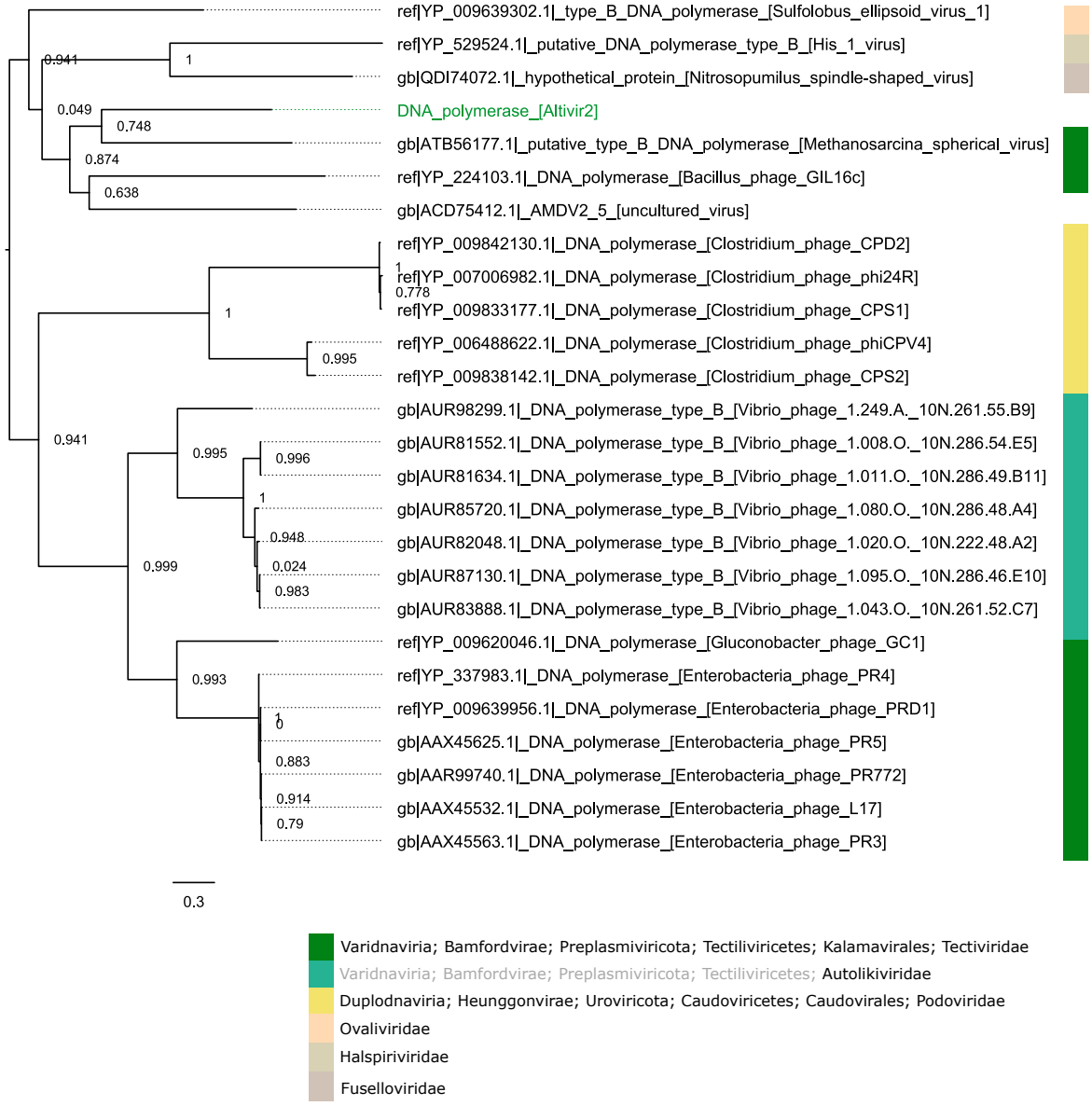
Supplementary Figure 4: Intergenomic similarities between *Ca.* Altiarchaeum virus Altivir genomes calculated by VIRIDIC (http://viridic.icbm.de /) [12]. ACLF=Alpena County Library Fountain, BF=biofilm, HURL=Horonobe Underground Research Laboratory, MSI=Mühlbacher Schwefelquelle Isling.



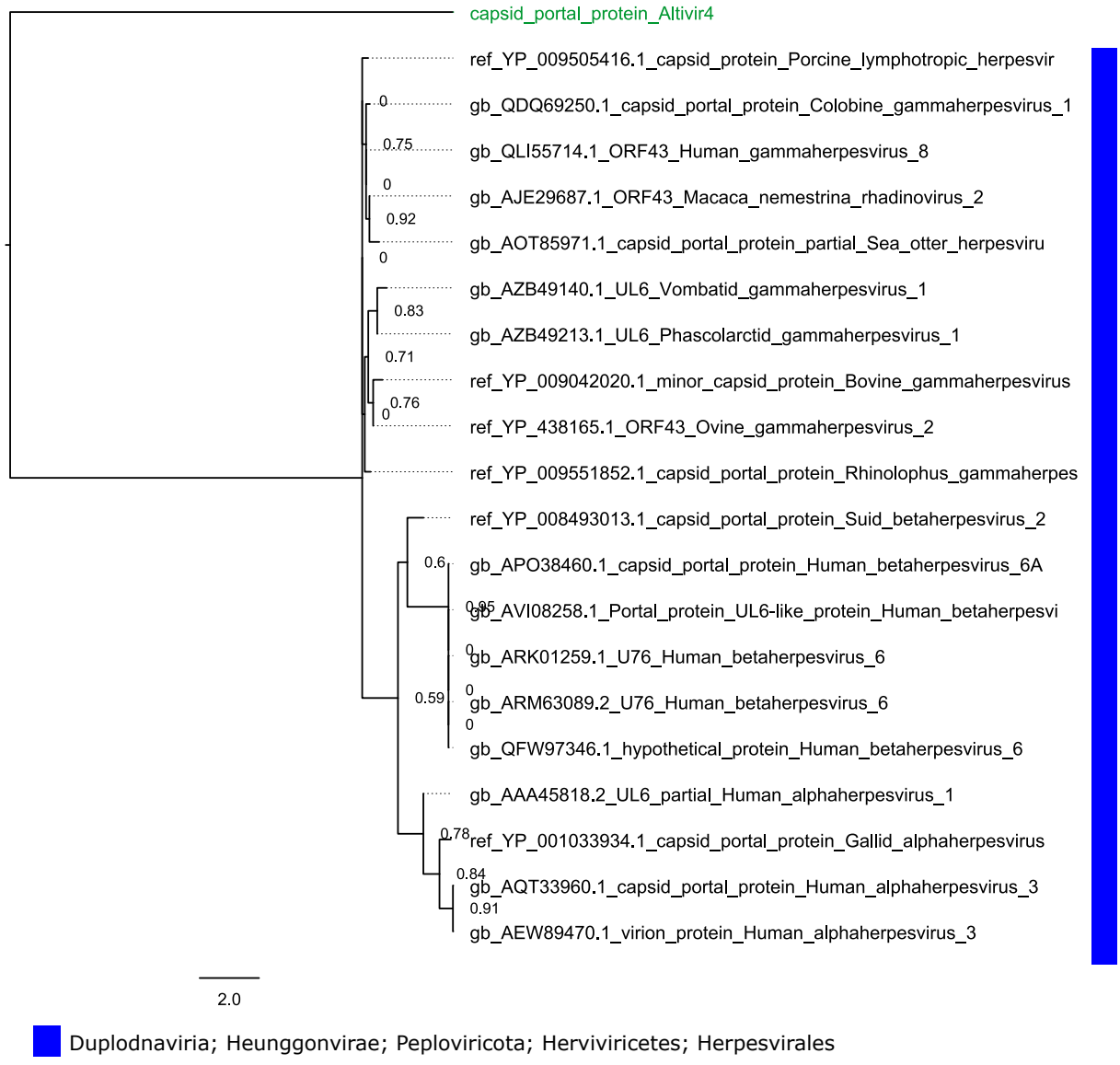
Supplementary Figure 5: Viral clusters of Altivir_1, _2, and _6 and their position in the viral network. Altivir_1_MSI genomes form a cluster with Altivir_6_ACLF while Altivir_2_MSI genomes form a cluster on their own. The network shows how genomes cluster according to similarities with the database "ProkaryoticViralRefSeq94-Merged" [13] after using vConTACT v.0.9.11 [14, 15]. Nodes and edges represent viral genomes and genome similarities, respectively. Visualization was done in Cytoscape v.3.7.2 [16]. All remaining genomes of *Ca.* Altiarchaeum viruses were excluded as unclustered singletons. ACLF=Alpena County Library Fountain, BF=biofilm, MSI=Mühlbacher Schwefelquelle Isling.



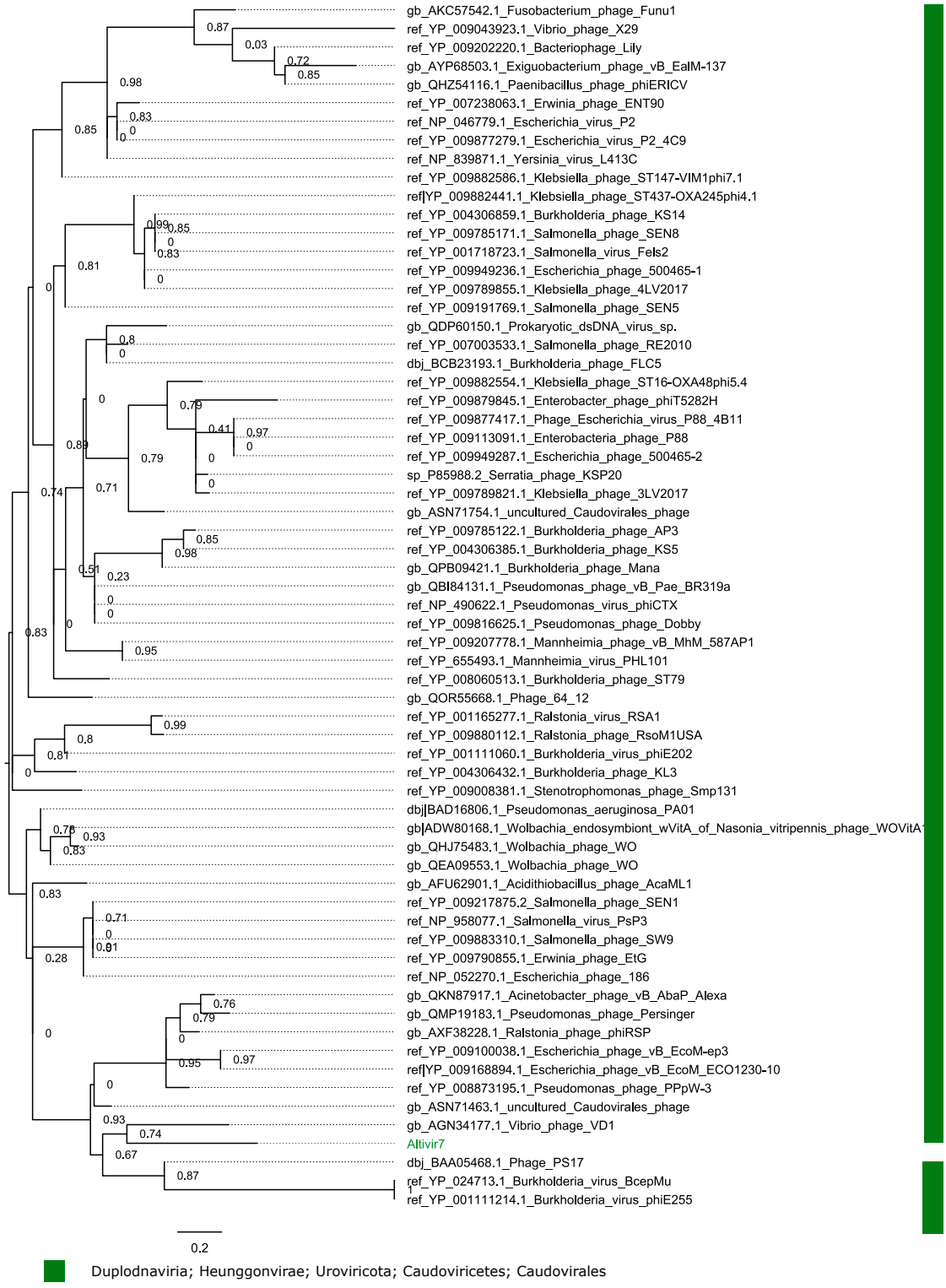
Supplementary Figure 6: Phylogenetic tree for the DNA polymerase of Altivir_2_MSI and related proteins. Internal branch labels represent branch support values, which have been calculated using the approximate likelihood-ratio test. The tree was rooted at midpoint. For further details, see Materials and Methods section.



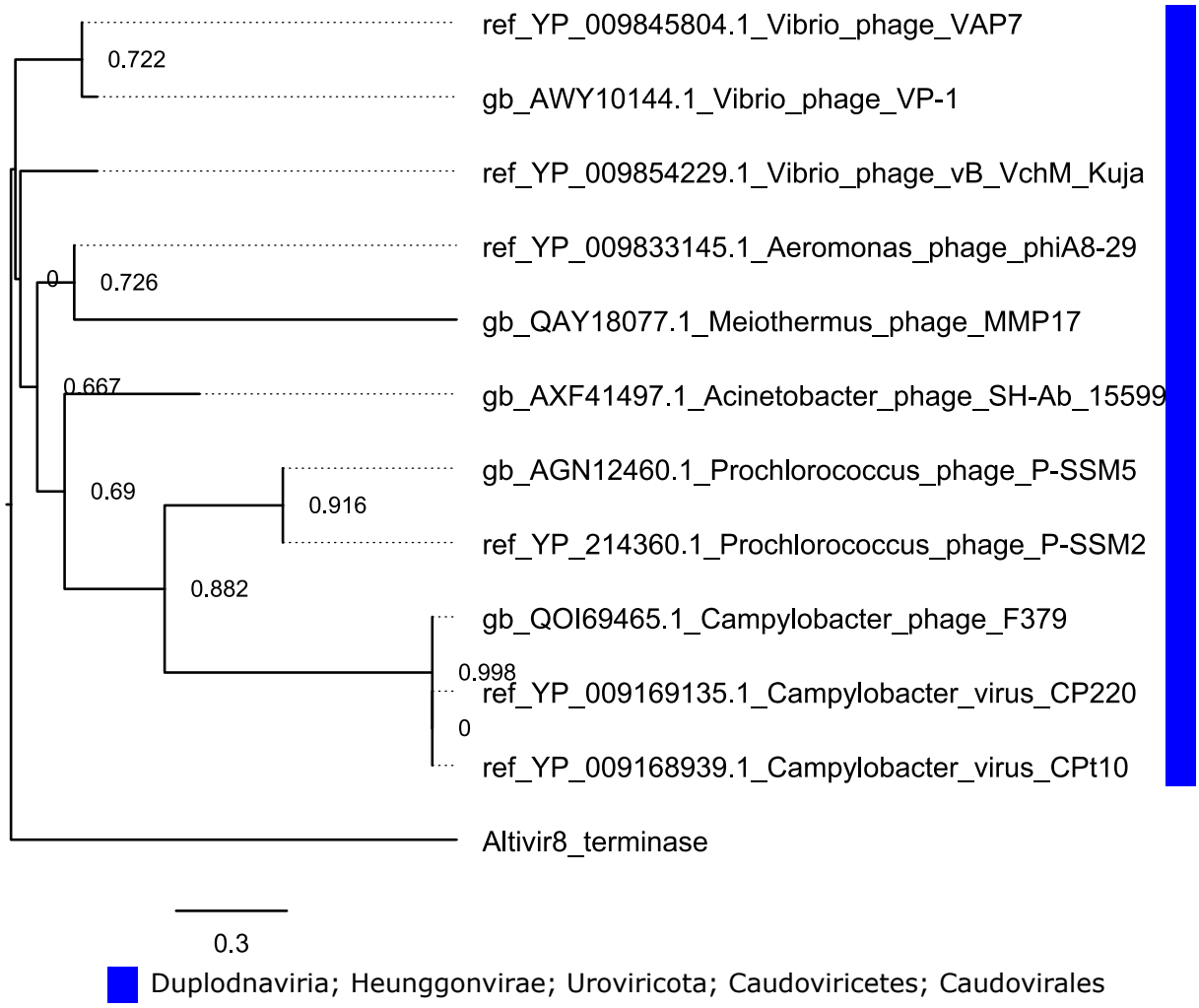
Supplementary Figure 7: Phylogenetic tree of the capsid portal protein of Altivir_4_ACLF and related proteins. Internal branch labels represent branch support values, which have been calculated using the approximate likelihood-ratio test. The tree was rooted at midpoint. For further details, see Materials and Methods section.



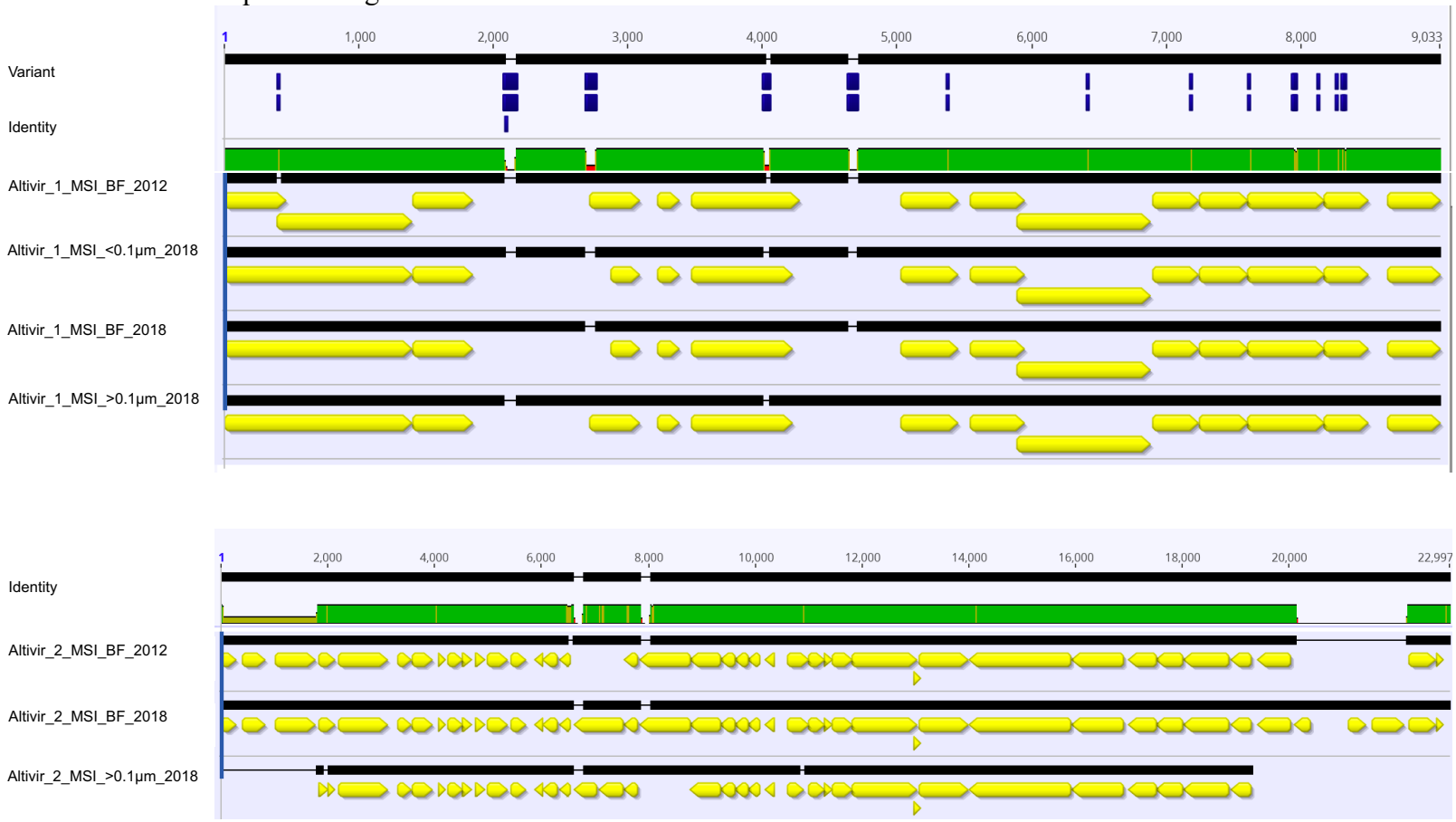
Supplementary Figure 8: Phylogenetic tree for the major tail tube protein of Altivir_7_ACLF and related proteins. Internal branch labels represent branch support values, which have been calculated using the approximate likelihood-ratio test. The tree was rooted at midpoint. For further details, see Materials and Methods section.



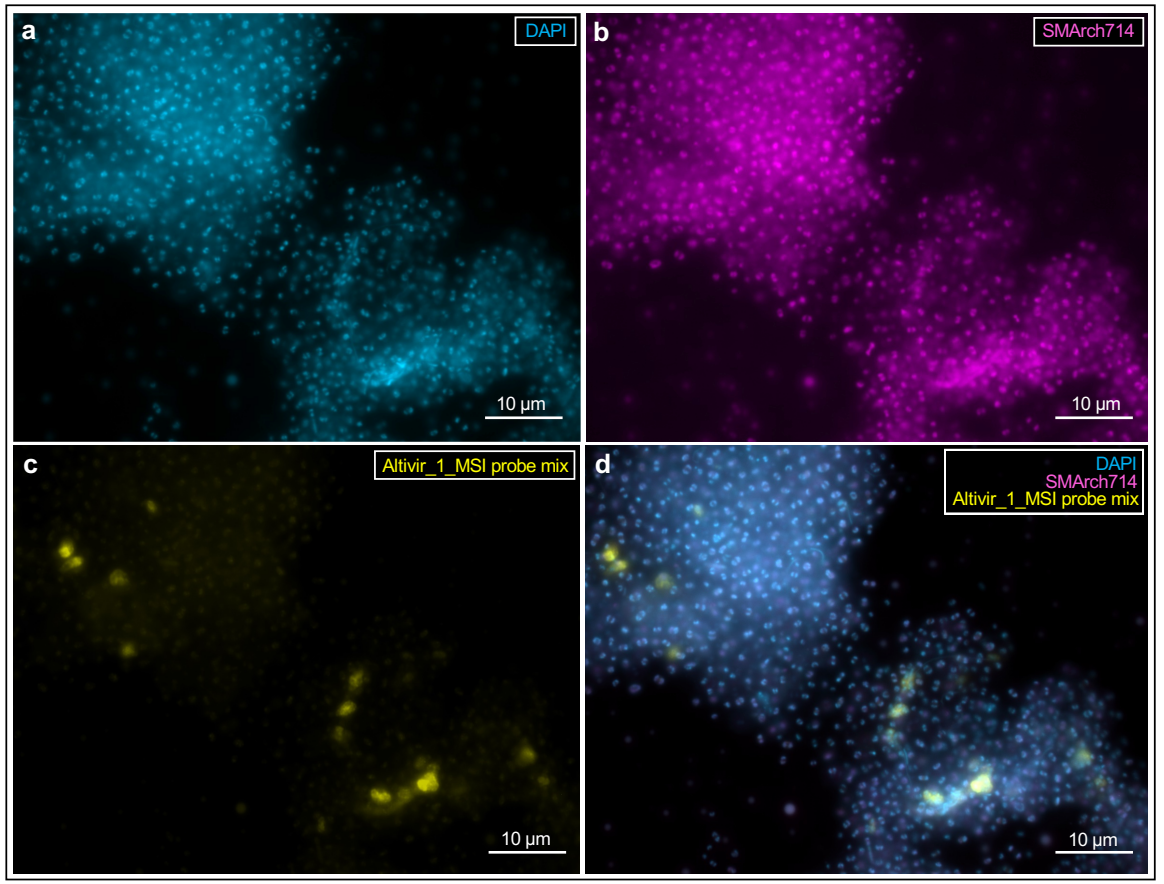
Supplementary Figure 9: Phylogenetic tree for the terminase protein of Altivir_8_HURL and related proteins. Internal branch labels represent branch support values, which have been calculated using the approximate likelihood-ratio test. The tree was rooted at midpoint. For further details, see Materials and Methods section.



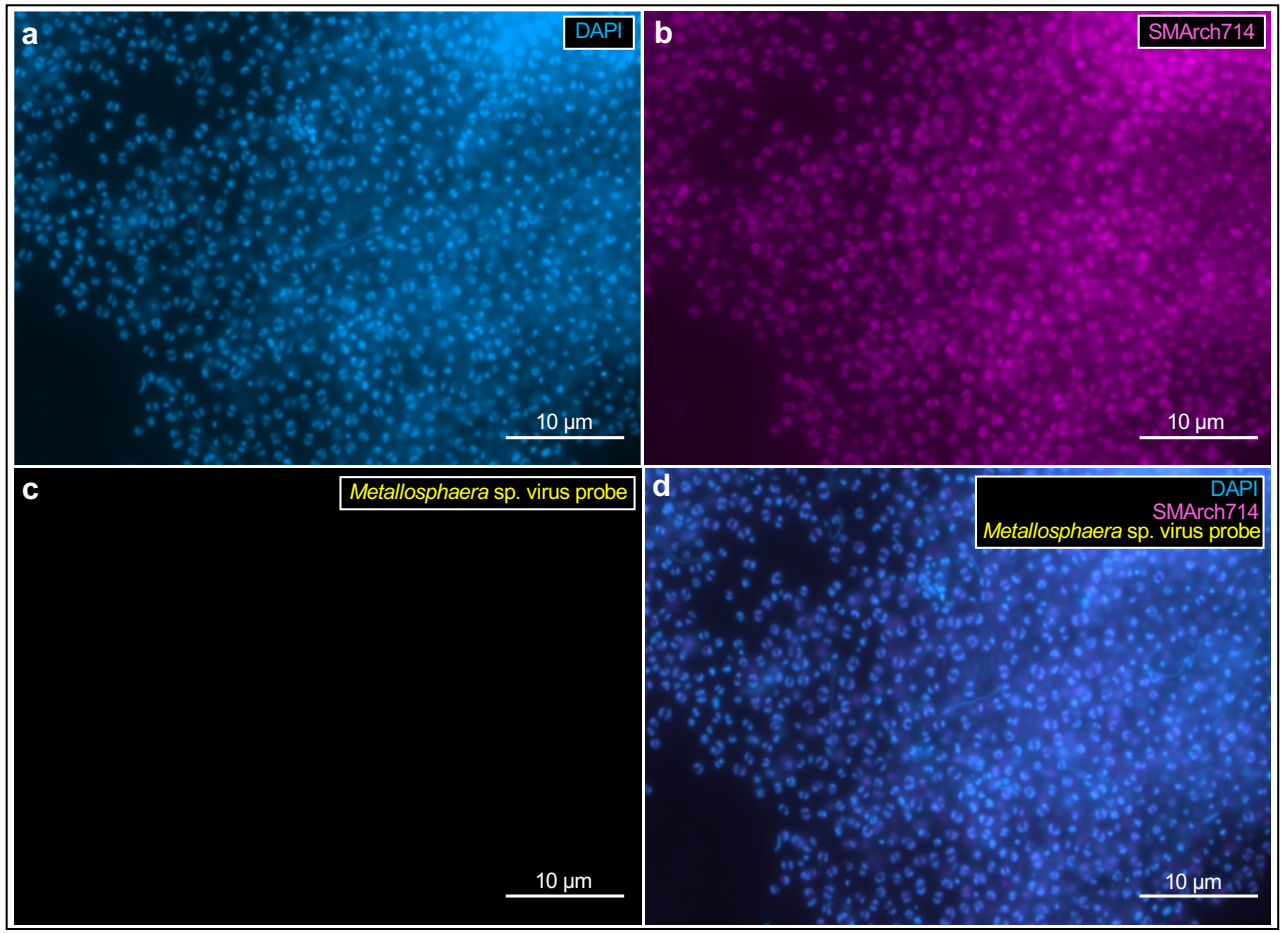
Supplementary Figure 10: Development of coding sequences (CDS) from 2012 and 2018 samples for Altivir_1_MSI (upper panel) and Altivir_2_MSI (lower panel). Variant regions can only be shown for Altivir_1_MSI due to the huge gaps in beginning and end of the Altivir_2_MSI alignments. Alignments were performed using MUSCLE [3], and variant calling was performed with default settings of Geneious Prime version 11.1.5 [17]. MSI=Mühlbacher Schwefelquelle Isling.



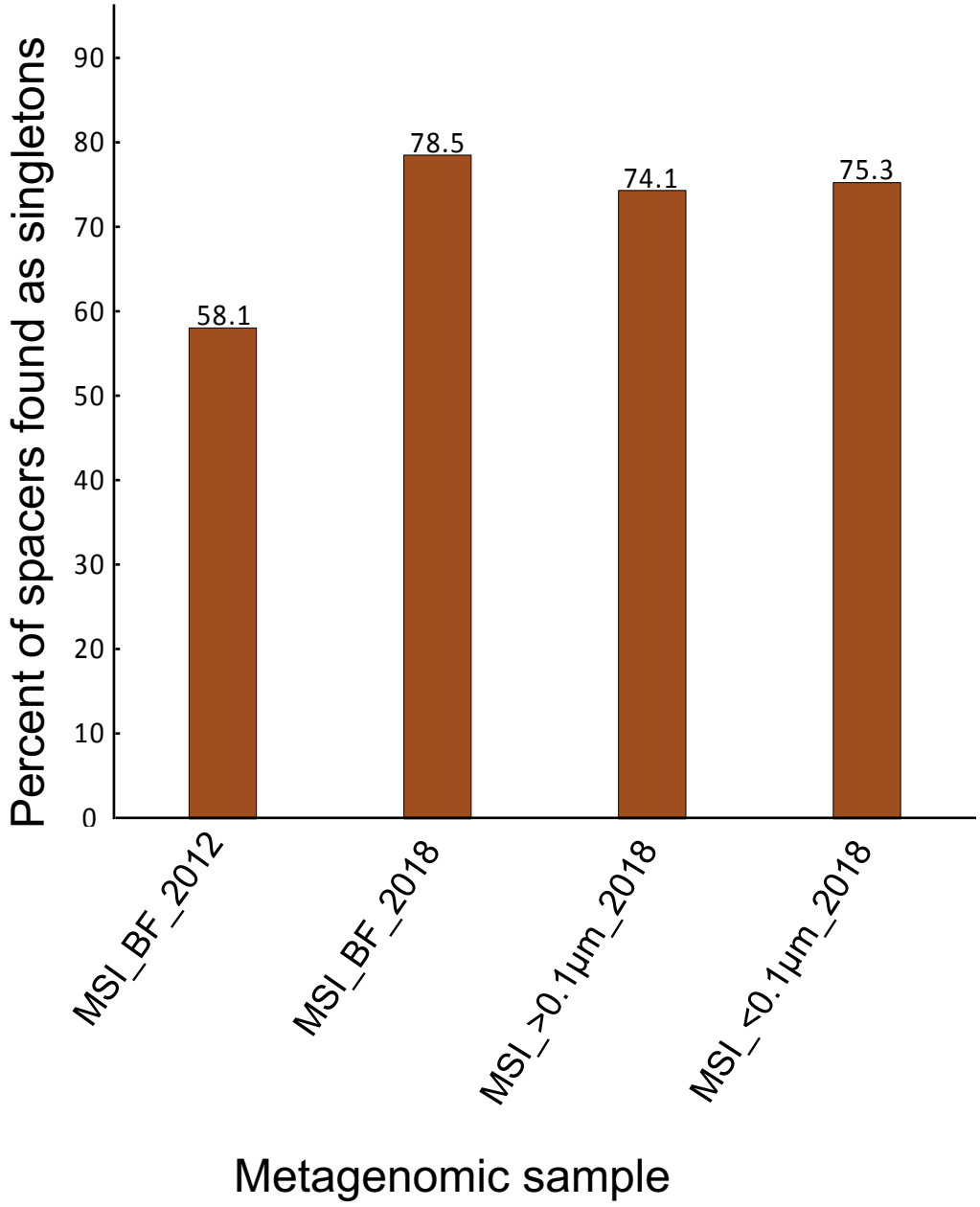
Supplementary Figure 11: VirusFISH performed on Altiarchaeota biofilms (BF) for visualization of viral infections caused by Altivir_1_MSI. For virusFISH, a chemically synthesized probe mix targeting Altivir_1_MSI was used. BF material was analyzed with A) DAPI (blue, cells), B) ATTO 488 (purple, 16S rRNA signal) and C) Alexa 594 (yellow, viral genomes of Altivir_1_MSI) and D) merged. VirusFISH experiments were highly reproducible: Since we established virusFISH for this project in 2019, we have tested the chemically synthesized probe mix targeting Altivir_1_MSI 70 times in total and the Metallosphaera sp. virus probe (as negative control) 66 times in total. The testing was always carried out on Altiarchaea biofilm samples from MSI site. Scale bars: 10 µm.



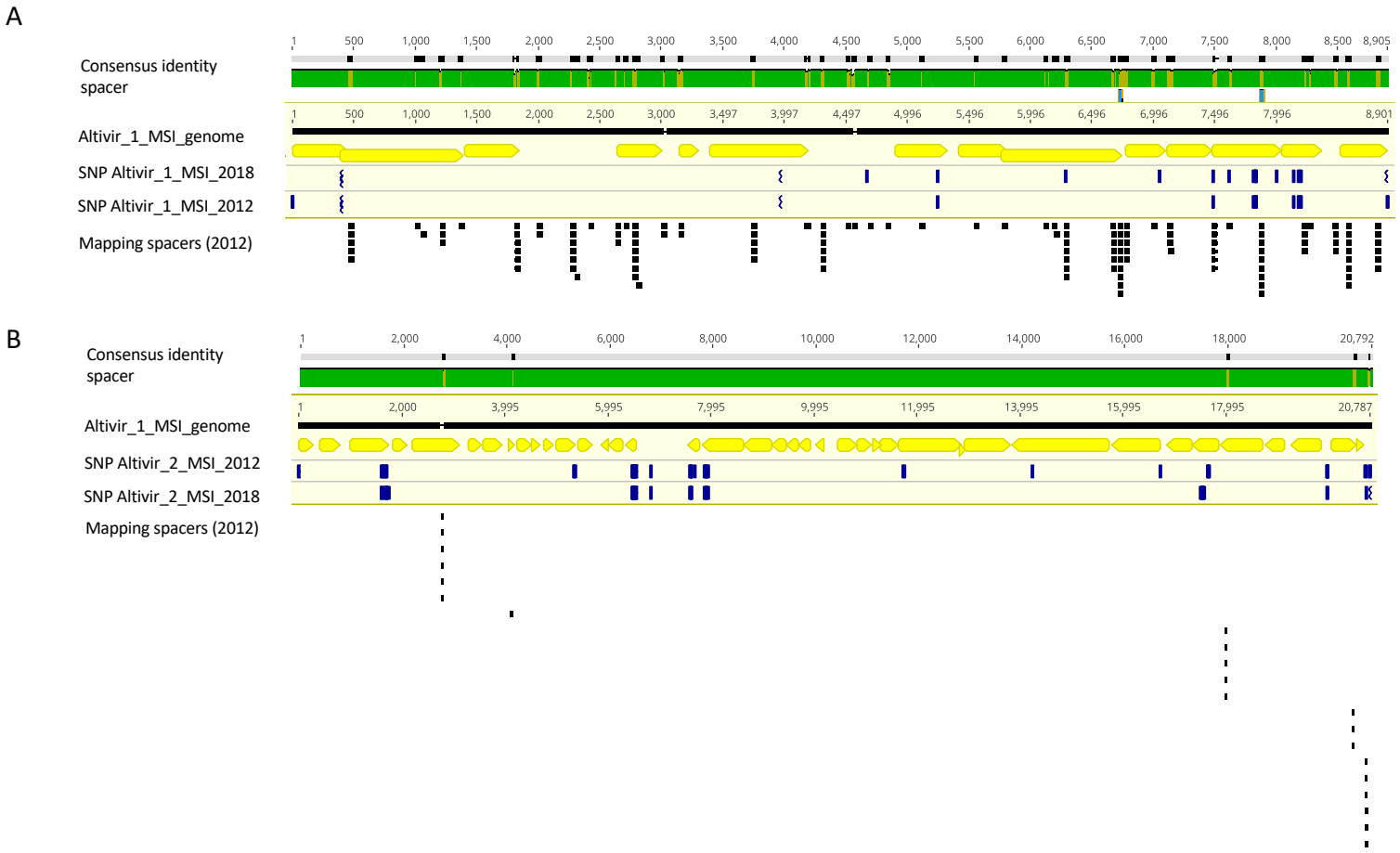
Supplementary Figure 12: A chemically synthesized *Metallosphaera* sp. virus probe was used as a non-matching probe for virusFISH showing no viral infections within an altiarchaeotal biofilm (BF). BF material was analyzed with A) DAPI (blue, cells), B) ATTO 488 (purple, 16S rRNA signal) and C) Alexa 594 (yellow) and merged (D). For a statement regarding reproducibility please see legend of Supplementary Figure 11. Scale bars: 10 µm.



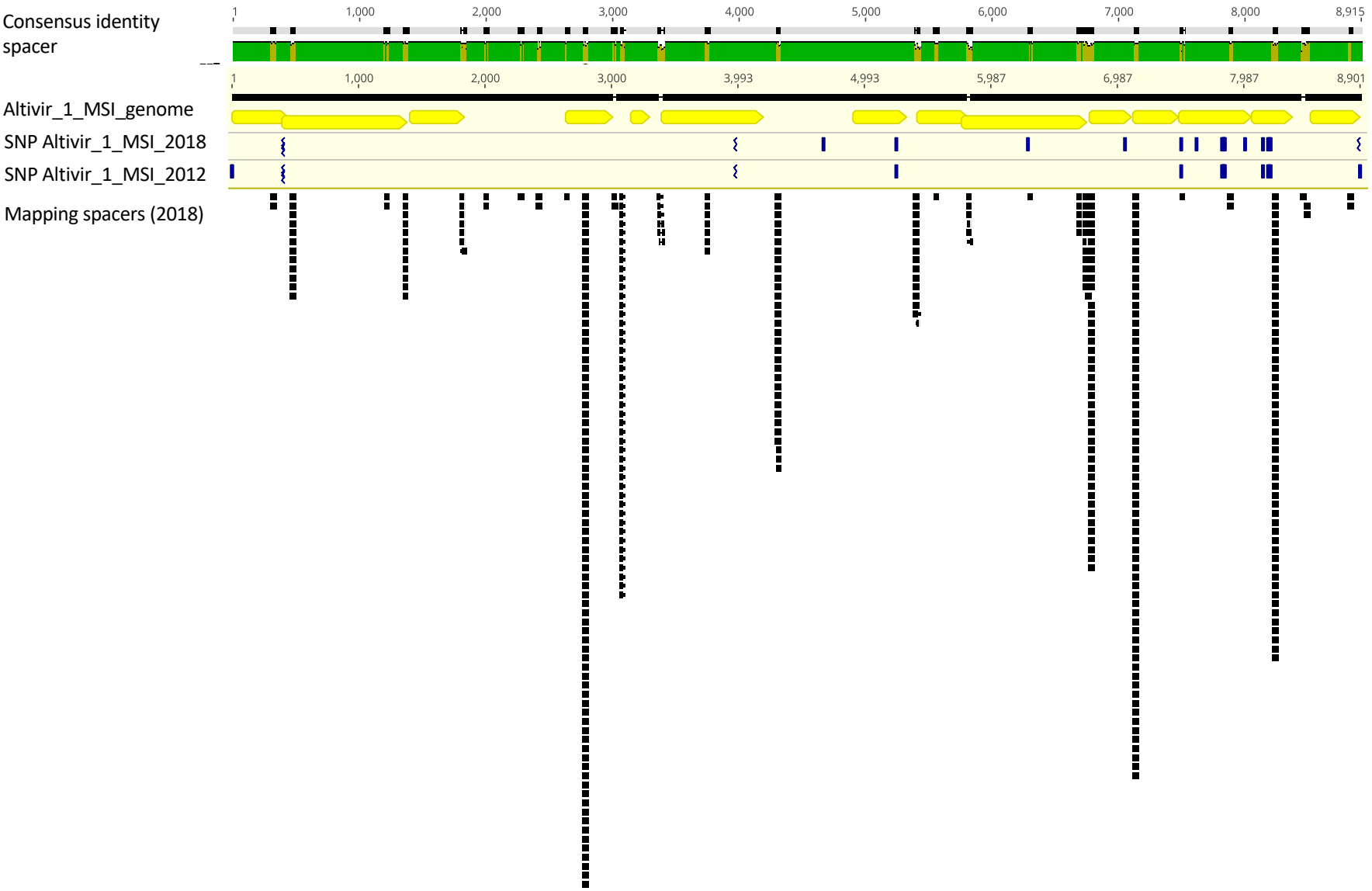
Supplementary Figure 13: Bar graph depicting the number of spacers found as singletons compared to the total number of different spacers per sample from the MSI site. An increase by ~20% from 2012 to 2018 is identified, which indicates a diversification of the CRISPR array in Altiarchaeota and of their respective strains. BF=biofilm, MSI=Mühlbacher Schwefelquelle Isling, CRISPR=Clustered regularly interspaced short palindromic repeat.



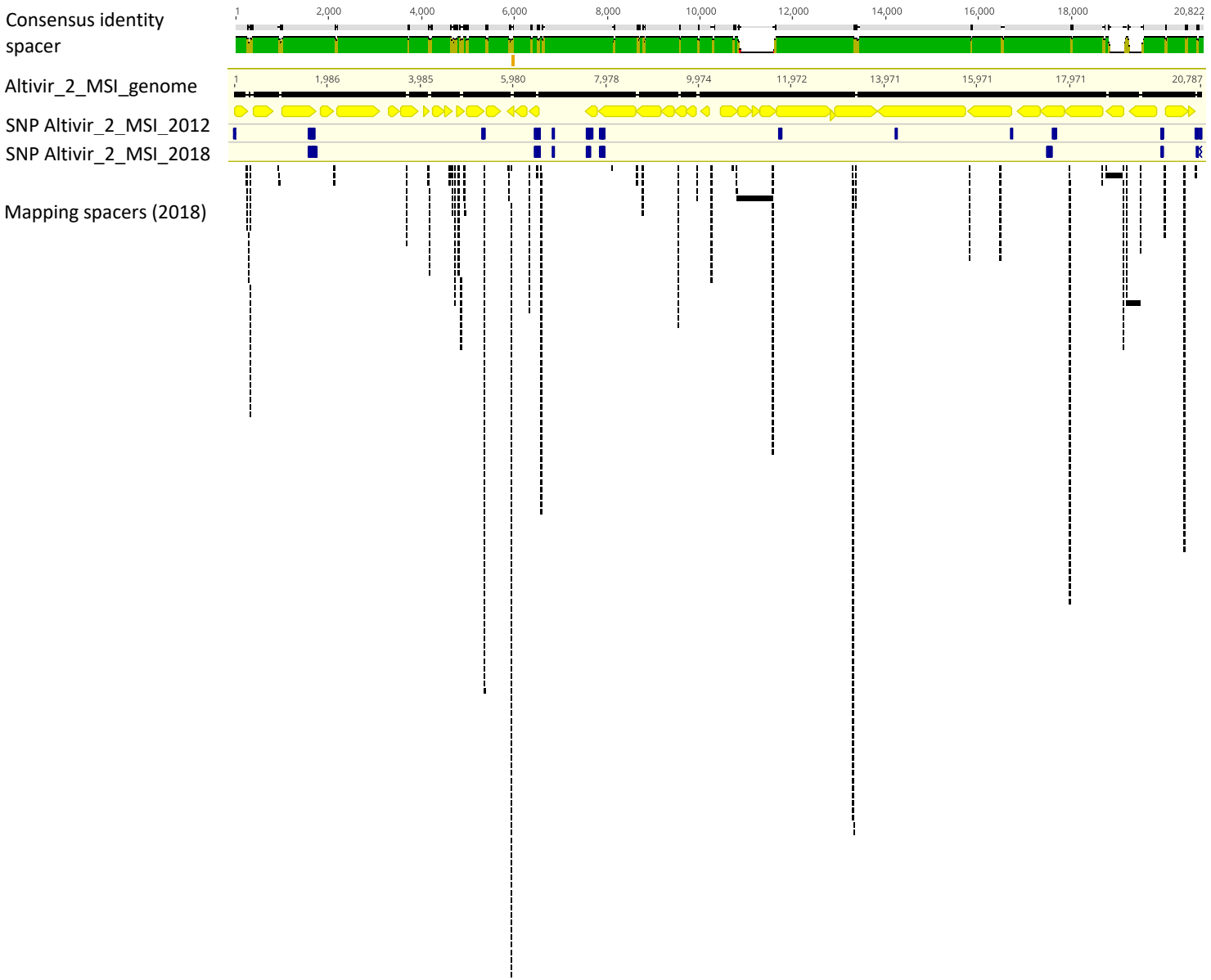
Supplementary Figure 14: Spacer mapping to predicted viral genomes and variants therein. A) Spacers extracted from MSI_BF_2012 metagenomes mapped against genomes of Altivir_1_MSI_BF_2012 and B) Altivir_2_MSI_BF_2012. C) Spacers extracted from MSI_BF_2018 metagenomes mapped against genomes of Altivir_1_MSI_BF_2012 and D) Altivir_2_MSI_BF_2012. Variants (abbreviated to SNP) are based on read mapping from reads of MSI_BF_2012 and MSI_BF_2018 samples (see Supplementary Data 2) and coding regions (in yellow) are shown. Variant calling was performed in default settings of Geneious Prime version 11.1.5 [17]. Mapping of paired reads to viral genomes was conducted using Bowtie 2 sensitive mode [18]. BF=biofilm, MSI=Mühlbacher Schwefelquelle Isling, SNP=single-nucleotide polymorphism.



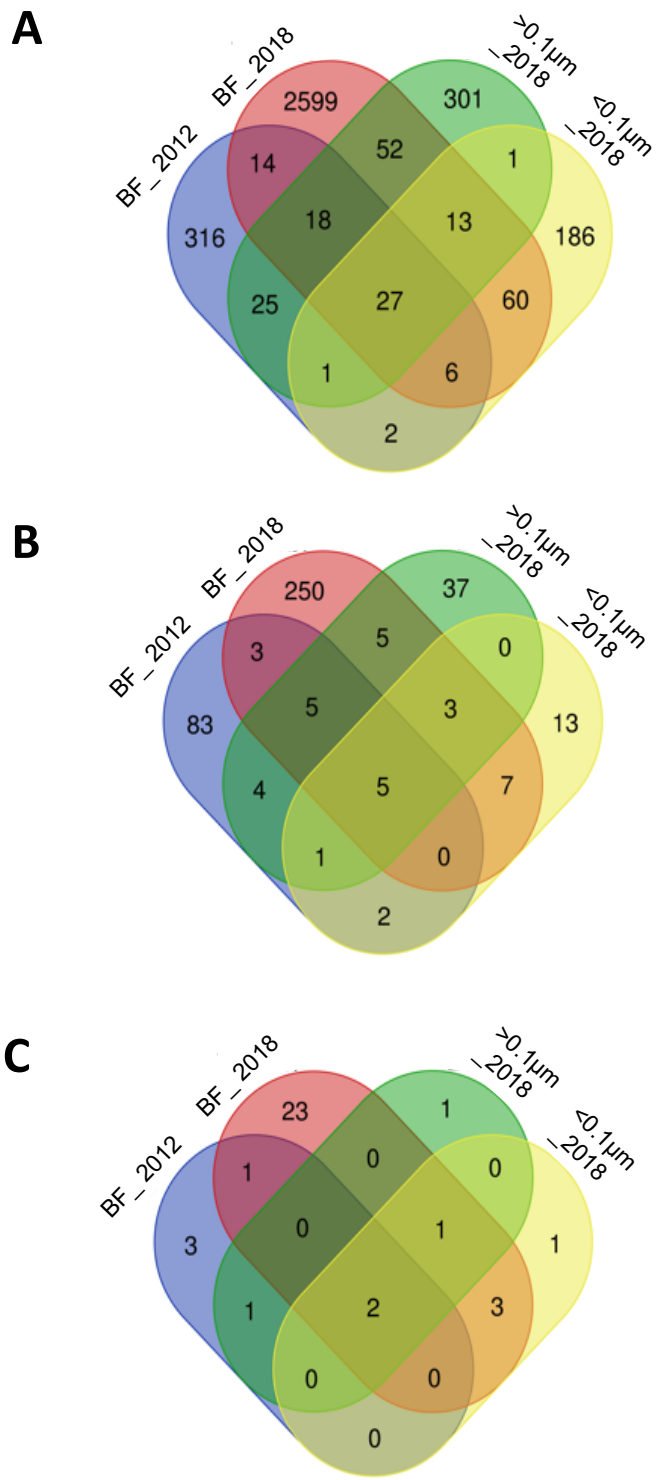
C


Consensus identity
spacer
1
1,000
2,000
3,000
4,000
5,000
6,000
7,000
8,000
8,915
1
1,000
2,000
3,000
3,993
4,993
5,987
6,987
7,987
8,901
Altivir_1_MSI_genome
SNP Altivir_1_MSI_2018
SNP Altivir_1_MSI_2012
Mapping spacers (2018)

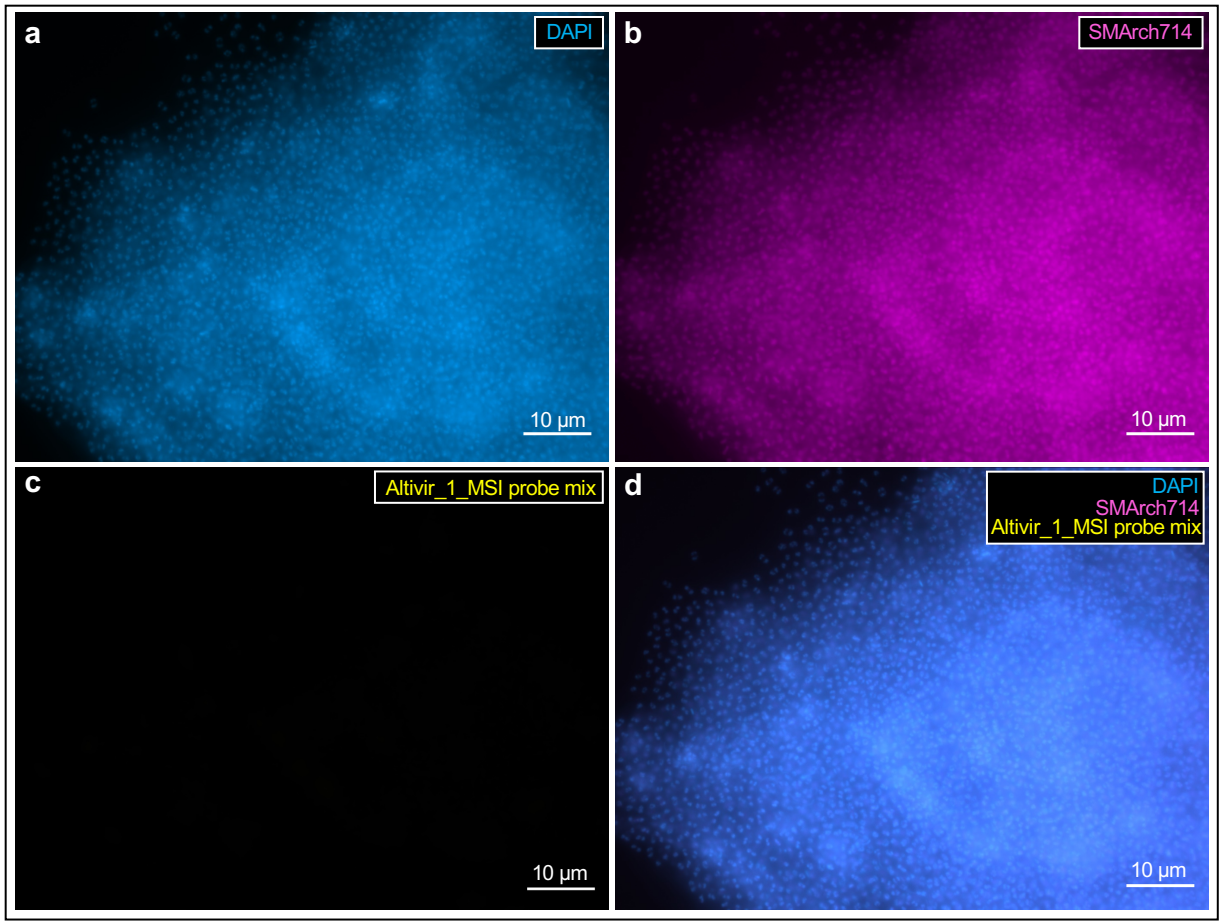
D


Consensus identity spacer
Altivir_2_MSI_genome
SNP Altivir_2_MSI_2012
SNP Altivir_2_MSI_2018
Mapping spacers (2018)
1
2,000
4,000
6,000
8,000
10,000
12,000
14,000
16,000
18,000
20,822
1
1,986
3,985
5,980
7,978
9,974
11,972
13,971
15,971
17,971
20,787

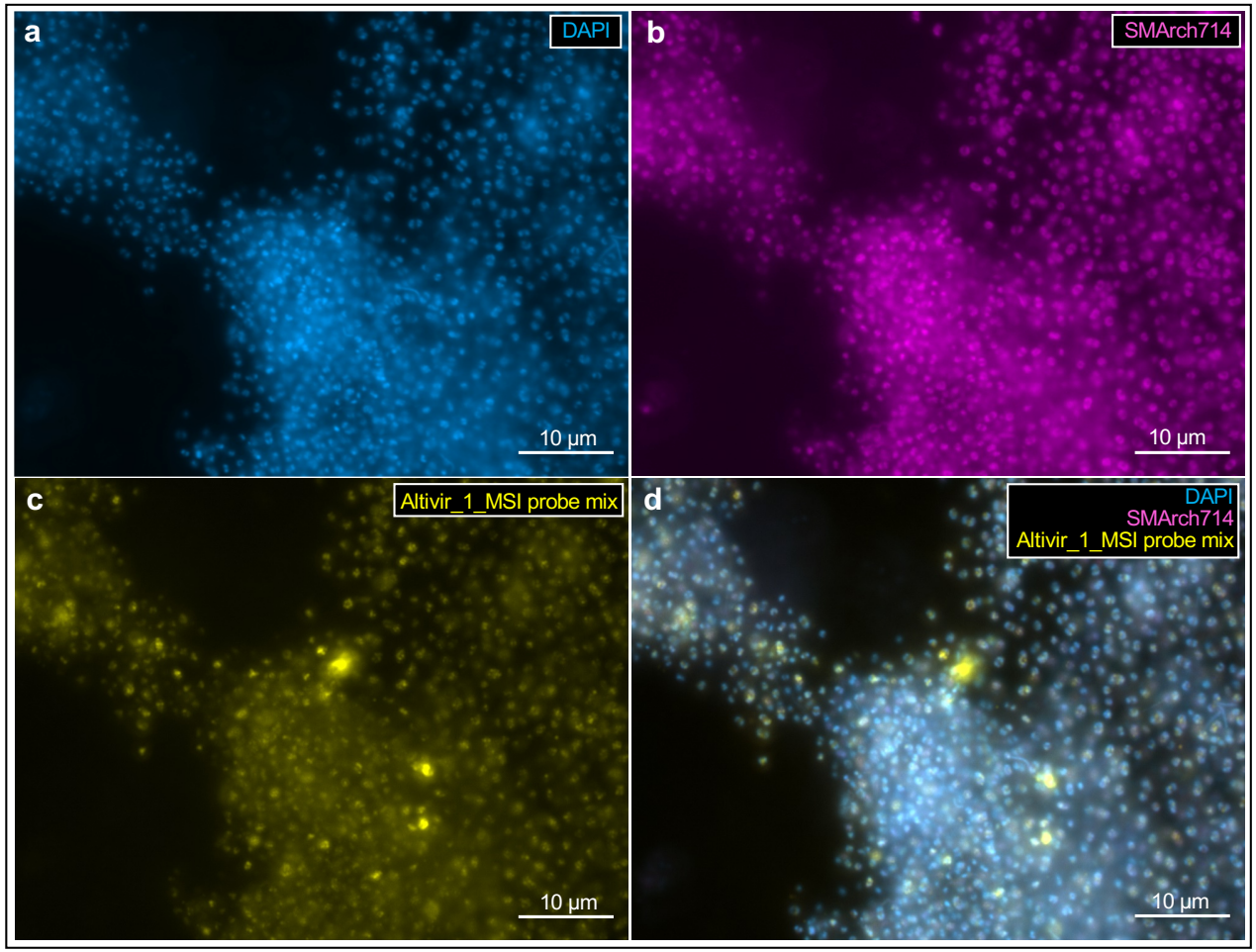
Supplementary Figure 15: Venn diagrams for shared spacer clusters between samples and years for A) total clusters, B) spacer clusters matching Altivir_1_MSI, and C) spacer clusters matching Altivir_2_MSI. The number of shared clusters might deviate from Figure 4, which show read-normalized spacer abundances (rel. abundances). Diagrams were constructed using the UGent webtool (http://bioinformatics.psb.ugent.be/webtools/Venn/). Colors indicate different samples as follows: blue=BF_2012, red=BF_2018, green=>0.1 µm_2018, yellow=<0.1 µm_2018; BF=biofilm.



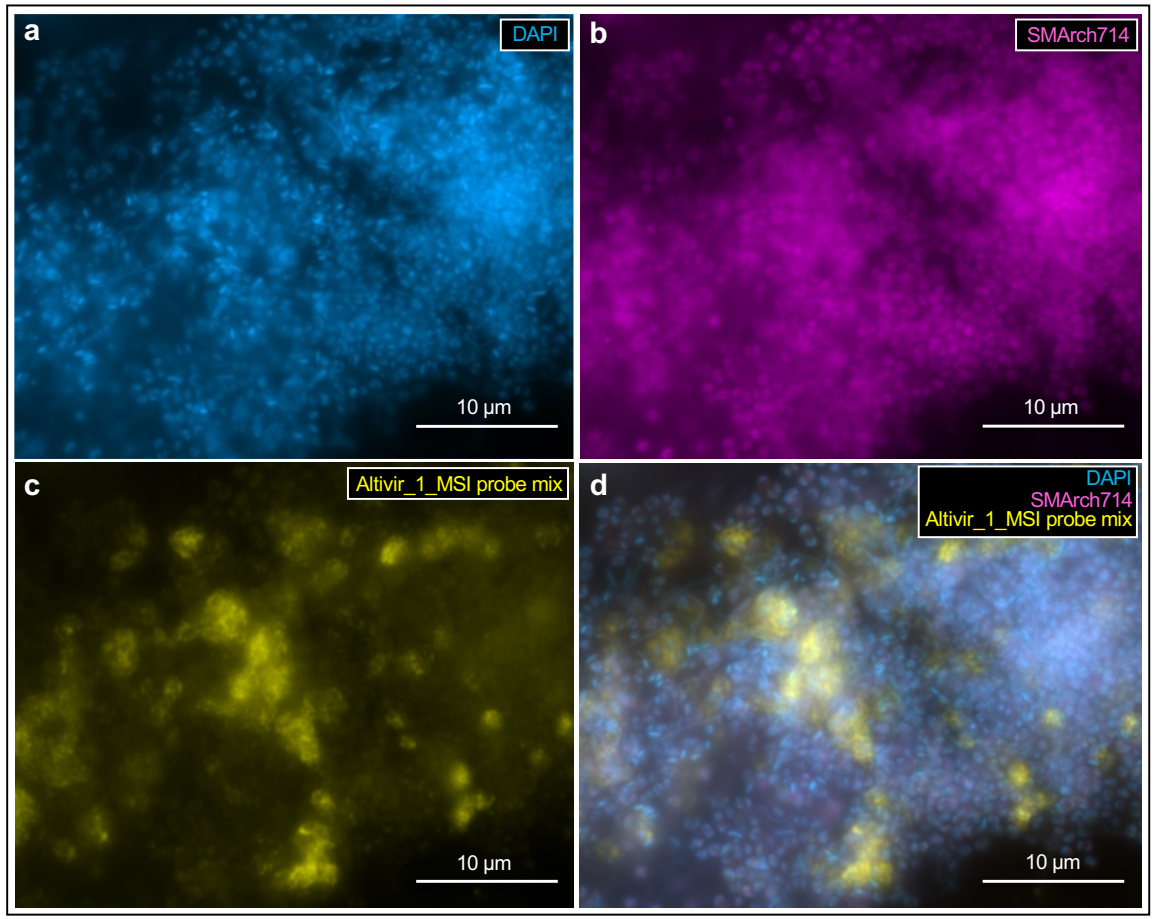
Supplementary Figure 16: VirusFISH of a dense Altiarchaeota biofilm (BF) flock showing no detectable infection by Altivir_1_MSI. Based on our enumeration, the majority of Altiarchaeota BF were infected by Altivir_1_MSI, however two out of seventeen BF flocks hybridized with the Altivir_1_MSI probe mix showed no infections at all. BF material was analyzed with A) DAPI (blue, cells), B) ATTO 488 (purple, 16S rRNA signal) and C) Alexa 594 (yellow, viral genomes of Altivir_1_MSI) and merged (D). For a statement regarding reproducibility please see legend of Supplementary Figure 11. Scale bars: 10 µm.



Supplementary Figure 17: EPI-fluorescence micrograph illustrating a heavily infected flock caused by Altivir_1_MSI. For virusFISH, a chemically synthesized probe mix targeting Altivir_1_MSI was used. Biofilm material was analyzed with A) DAPI (blue, cells), B) ATTO 488 (purple, 16S rRNA signal) and C) Alexa 594 (yellow, viral genomes of Altivir_1_MSI) and merged. For a statement regarding reproducibility please see legend of Supplementary Figure 11. Scale bars: 10 µm.



Supplementary Figure 18: EPI-fluorescence micrograph showing a heavily infected flock containing some rod-shaped bacteria potentially benefitting from lysing Altiarchaeota cells. For virusFISH, a chemically synthesized probe mix targeting Altivir_1_MSI was used. Biofilm material was analyzed with A) DAPI (blue, cells), B) ATTO 488 (purple, 16S rRNA signal) and C) Alexa 594 (yellow, viral genomes of Altivir_1_MSI) and merged (D). For a statement regarding reproducibility please see legend of Supplementary Figure 11. Scale bars: 10 µm. Scale bars: 10 µm.



Supplementary References


1. Rudolph C, Moissl C, Henneberger R, Huber R. Ecology and microbial structures of archaeal/bacterial strings-of-pearls communities and archaeal relatives thriving in cold sulfidic springs. *FEMS Microbiol Ecol* **50**, 1-11 (2004).

2. Nurk S, Meleshko D, Korobeynikov A, Pevzner PA. metaSPAdes: a new versatile metagenomic assembler. *Genome Res* **27**, 824-834 (2017).

3. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**, 1792-1797 (2004).

4. Hofacker IL. Vienna RNA secondary structure server. *Nucleic Acids Res* **31**, 3429-3431 (2003).

5. Roux S, Enault F, Hurwitz BL, Sullivan MB. VirSorter: mining viral signal from microbial genomic data. *PeerJ* **3**, e985 (2015).

6. Ren J, Ahlgren NA, Lu YY, Fuhrman JA, Sun F. VirFinder: a novel k-mer based tool for identifying viral sequences from assembled metagenomic data. *Microbiome* **5**, 69 (2017).

7. Marz M*, et al.* Challenges in RNA virus bioinformatics. *Bioinformatics* **30**, 1793-1799 (2014).

8. Crits-Christoph A*, et al.* Functional interactions of archaea, bacteria and viruses in a hypersaline endolithic community. *Environ Microbiol* **18**, 2064-2077 (2016).

9. Eßer S. Dynamics of Altiarchaeota and their putative viruses in a deep terrestrial subsurface ecosystem. In: *Department of Chemistry*). University of Duisburg-Essen (2019).

10. Dabney A, Storey JD, Warnes G. qvalue: Q-value estimation for false discovery rate control. *R package version* **1**, (2010).

11. Team RC. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. (2019).

12. Moraru C, Varsani A, Kropinski AM. VIRIDIC-A novel tool to calculate the intergenomic similarities of prokaryote-infecting viruses. *Viruses* **12**, (2020).

13. Brister JR, Ako-Adjei D, Bao Y, Blinkova O. NCBI viral genomes resource. *Nucleic Acids Res* **43**, D571-577 (2015).

14. Bin Jang H*, et al.* Taxonomic assignment of uncultivated prokaryotic virus genomes is enabled by gene-sharing networks. *Nat Biotechnol* **37**, 632-639 (2019).

15. Bolduc B, Jang HB, Doulcier G, You ZQ, Roux S, Sullivan MB. vConTACT: an iVirus tool to classify double-stranded DNA viruses that infect Archaea and Bacteria. *PeerJ* **5**, e3243 (2017).

16. Shannon P*, et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* **13**, 2498-2504 (2003).

17. Kearse M*, et al.* Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647-1649 (2012).

18. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods* **9**, 357-359 (2012).