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Draft Genome Sequence of Medusavirus stheno, a new member of "Medusaviridae" Isolated from the Tatakai River of Uji, Japan

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Abstract

"Medusaviridae" is a proposed family of large DNA viruses so far represented by a sole virus isolated from a hot spring. In the present study, we report the isolation and genome sequencing of a new member of this family, medusavirus stheno, discovered from a freshwater sample with *Acanthamoeba castellanii* coculture.

The founding member of "Medusaviridae" of the phylum *Nucleocytoviricota* was previously isolated from a hot spring in Japan (1). The new second member of this family, named medusavirus stheno, was isolated from water/soil samples from the Tatakai River, Uji, Japan.

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Samples were filtered with filter paper 43 (Whatman PLC) and 1.2-µm pore size Minisart® Syringe Filter (Sartorius). Ninety µL of solution (18 mL peptone yeast extract-glucose (PYG), 500 µL of amoeba cells) and 9.5 µL of filtered samples were added in a 96-well plate. After 7 days of culture (26°C), 10 µL of supernatant from each well showing delayed proliferation was mixed with 1 mL of PYG, 3 drops of amoeba culture solution in a 24-well plate. After 7 days, the supernatant from wells showing delayed proliferation was serially diluted down to 10^{-11} fold with PYG. Ten µL of each diluted solution was mixed with 90 µL of PYG medium (16 mL PYG and 300 µL amoeba cells) in a 96-well plate. After another 7 days of culture, wells showing a delayed growth with at least 10^{-6} fold dilution were considered to contain viruses without bacteria. Fresh amoeba cells were inoculated with supernatants from these wells in a 75 cm^3 culture flask. After two days, cells were harvested and centrifuged at 538 x g for 5 min twice at 26°C, then the supernatant was centrifugated at 8000 x g for 35 min at 4°C. The resulting pellet was resuspended with 1 mL of phosphate-buffered saline. Centrifugation and resuspension were repeated twice to obtain pellets containing the putative viral particles. Observation of virions in amoeba cells using transmission electron microscopy was performed as described previously (Fig. 1A) (2, 3).

DNA extraction was performed with NucleoSpin Tissue XS kit (Macherey-Nagel GmbH and Co. KG). Sequencing was performed using Nanopore MinION (Oxford Nanopore Technologies, Inc.), HiSeq X Ten (Illumina, Inc.) and MiSeq (Illumina, Inc.). Unicycler (with options SPAdes assembler, Pilon polisher) and Bandage were used for *de novo* hybrid assembly (4, 5), which produced a 362,811 bp contig. Coding sequences (CDSs) were predicted using Prodigal (6) and GeneWise v2.4.1 (7). Putative promoter motifs were identified using MEME version 5.1.1 with E-value < 10^{-50} (8). Homology searches (E-value < 10^{-5}) were performed against NCBI NR using BLASTP (9).

The contig was colinearly aligned with the original medusavirus genome (381 kb, the average nucleotide identity of 79.8%, Fig. 1B) and found to encode 429 CDSs, of which 349 (81%) had their best BLASTP hit in the original medusavirus. Like the original virus, the new medusavirus stheno encoded a complete set of histone domains (H1, H2A, H2B, H3, and H4), but, unlike the original one, H3 and H4 were found encoded in a fused single CDS (ORF_240). Two conserved sequence motifs were identified in

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the upstream regions of the CDSs (Table 1). These motifs were also found in the original medusavirus genome.

Data availability. Raw reads and the draft genome sequence of have been deposited in DDBJ (DRA010707) and GenBank (MW018138), respectively.

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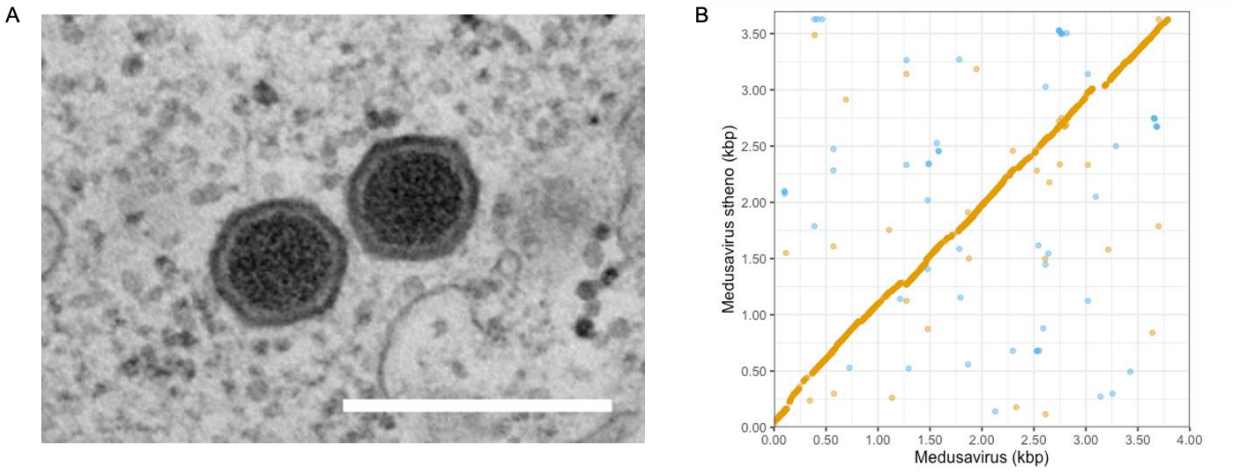
Figure Legends

Fig 1. Medusavirus stheno virions and geome comparison. (A) Observation of medusaivrus stheno virions by transmission electron microscopy (scale bar 400 nm). (B) Comparison of medusavirus genomes. Dot plot was generated using MUMmer version 3.23 (10).

Table Legends

Table 1. Sequence motifs identified in the upstream regions of medusaviruses.



Medusavirus stheno			Medusavirus		
Consensus sequence	Sites	E-value	Consensus sequence	Sites	E-value
SRCCAYATGAMBTCACATGGC	43	4.1e-162	GCCATRTGAVKTCATRTGGYSRSG	53	8.4e-183
VMMMAMADMAAMAAA	252	5.5e-130	VMAAMAAMARMAAMA	251	3.1e-146