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Rio Negro virophage: Sequencing of the near complete genome and transmission electron microscopy of viral factories and particles



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ABSTRACT

Rio Negro virophage (RNV) was co-isolated with a strain of mimivirus named sambavirus, from Brazilian Amazon. We report the near complete genome sequence of RNV, the first virophage isolated in Brazil. We also present new microscopical data demonstrating that RNV particles have similar dimensions to that described to sputnik virophages.

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Rio Negro virophage (RNV) was co-isolated in 2011 with a strain of mimivirus lineage A named sambavirus, from Rio Negro river, Brazilian Amazon.¹ Phylogenetic analyses based on RNV capsid gene revealed the clustering of RNV with sputnik virophages.¹ Hereby describe the nearly complete genome of RNV.

Sambavirus and RNV were co-cultured in *A. castellanii* monolayers at a multiplicity of infection of 0.01 during four days. The lysate was collected and centrifuged at 1400 *g* for 10 min. The resultant supernatant was consecutively passed through 0.8 and 0.2 μ m filters and ultracentrifuged at 90,000 *g* for 2 h in a sucrose cushion of 38%. The genome was extracted from the purified RNV sample and sequenced by Illumina MiSeq instrument (Illumina Inc., San Diego, CA, USA) with

the paired end application and assembled *de novo* using SPADES v. 3.5.0, followed by manual annotation based on genomes of Sputniks 1, 2 and 3 (GenBank, NCBI: EU606015.1, JN603369.1 and JN603370.1, respectively). Localizations within RNV genome mentioned in this work are therefore based on its alignment with these three sequences by Clustal W, MEGA 7.0.26.

RNV genome is a double-stranded DNA molecule of 18,145 bp encoding 20 putative open reading frames ranging from 330 to 2340 bp. This information added to the analyses of RNV genome, corroborate its close relationship with sputnik strains. The RNV and sputnik 2 strains present a highly similar gene content and genome synteny despite SNPs and insertions observed in both coding and non-coding regions.

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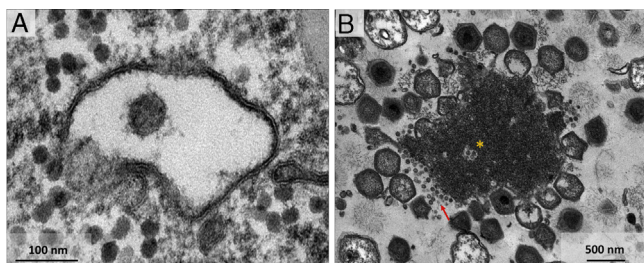


Fig. 1 – Transmission electron microscopy of RNV in *Acanthamoeba* cells co-infected with sambavirus. (A) RNV particle inside a vesicle at time 8 h post infection. (B) Sambavirus viral factory (asterisk) producing both sambavirus particles and RNV particles (arrow).

RNV presents a 49bp insertion at position 11,841 leading to a longer intergenic region between ORFs 14 and 15. This sequence is a repetition of the preceding 49 nucleotides except for a SNP at the 22nd position, where a C was replaced by a G. There are also SNPs at positions 16,075, 18,141 and a deletion at 18,145. At the position 18,016 there is the insertion of 3 guanines. Compared to other sputnik genomes, the last 244 bp of RNV genome are missing. The successful amplification of a PCR targeting this region in RNV indicates that it also presents this last fragment (data not shown). Since the best hit of RNV genome is sputnik 2, RNV was deposited in GenBank as Sputnik virophage 2 isolate Rio Negro (accession number MG676470).

Transmission electron microscopy of RNV purified particles showed 50–70 nm particles, as observed to other sputnik viruses (Fig. 1A). This is consistent with that expected, since RNV capsid gene is very similar to sputnik virophages orthologs. In addition to that demonstrated in our previous

work,¹ it was possible to visualize RNV particles being formed inside sambavirus viral factories, as demonstrated elsewhere^{2,3} (Fig. 1B).

In summary, RNV is a sputnik-like virophage isolated from Brazilian Amazon, with genomic and morphological characteristics very similar to those described to sputnik virophage group.

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