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A Narnavirus-Like Element from the Trypanosomatid Protozoan Parasite Leptomonas seymouri

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Genome sequences were determined for a novel RNA virus, Leptomonas seymouri Narna-like virus 1 (LepseyNLV1). A 2.9-kb segment encodes an RNA-dependent RNA polymerase (RdRp), while a smaller 1.5-kb segment showed no database search matches. This is the first report of bisegmented Narnaviridae from insect trypanosomatids.

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From in silico screens of metatranscriptomic datasets for protozoan viruses, we encountered within archives of Leptomonas seymouri (order Kinetoplastida, Excavata eukaryotic supergroup) a hit to members of the Narnaviridae (1–3), which had not been previously identified in trypanosomatid protozoa. From a total of 300 million reads, 0.22% were assembled into a contig of 2,914 nucleotides (nt), predicting a 932-amino acid (aa) protein that bore motifs typical of narnaviral RNA-dependent RNA polymerase (RdRps) (1–3). As this was absent from previous assemblies (4), we isolated RNA from the L. seymouri strain ATCC 30220 using TRIzol reagent (Thermo Fisher), followed by digestion with DNase I (Thermo Fisher), purification with RCC-25 kit (Zymo Research), and digestion by S1 nuclease (Thermo Fisher).

Two cytoplasmic double-stranded RNAs (dsRNAs) of ~3 and ~1.5 kb were visualized after agarose gel electrophoresis (4, 5), which were eluted and used for generation of cDNA. Reverse transcriptase-PCR (RT-PCR) tests showed that the 3-kb band corresponded to the 2,914-nt RdRp contig, cDNA for the 1.5-kb band was inserted into bacterial vectors and sequenced (6), identifying a 1,455-nt contig in the metatranscriptomic assembly, which was confirmed by RT-PCR amplification. Blast-based searches did not yield any matches in the sequence databases tested.

Proventially, we term these elements the L. seymouri Narna-like virus 1 (LepseyNLV1) L and S segments; however, their functional association remains to be proven. Some data suggest that both segments are unstable during culture, as sensitive RT-PCR tests of this strain acquired independently from another laboratory did not reveal them (the authenticity of this strain was confirmed by sequence of two nuclear genes, GAPDH and PTR1). As in other multisegmented RNA viruses, loss of the RdRp would be expected to result in the loss of the remaining segments.

Viruses in the family Narnaviridae (“naked RNA”) lack capsids or envelopes and do not form infectious viral particles. They reside in the cytosol as an RNA-protein complex containing one single-stranded RNA segment of about 3 kb, with a single open reading frame (ORF) encoding the RdRp (1–3). Two genera are recognized; unlike cytosolic narnaviruses, mitoviruses are found in the mitochondrion of fungi and translated using the mitochondridial genetic code (2). Phylogenetic comparisons of the L segment RdRp with other Narnaviridae place it firmly within Narnavirus as a new species, since the overall amino acid divergence exceeds 80%. Interestingly, the trisegmented Ourmiavirus spp. show a closer relationship to Narnavirus than to Mitovirus (2, 7), suggesting that the bisegmented LepseyNLV1 exhibits some characteristics intermediate between Narnavirus and Ourmiavirus.

Leptomonas is a monoxenous kinetoplastid parasite of insects, and related parasites are widespread in insects around the world (8). Interestingly, L. seymouri has been repeatedly isolated from patients infected by Leishmania donovani (9), although in vitro, it is unable to survive in mammalian macrophages (4, 10). Because viruses within the related protozoan Leishmania guyanensis have been associated with elevated pathogenicity in animal models (11), LepseyNLV1 potentially contributes to the pathogenicity of Leptomonas-Leishmania coinfections (9).

Nucleotide sequence accession numbers. The genome sequences of the LepseyNLV1 L and S segments have been deposited in GenBank under accession numbers KU935604 and KU935605.

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