AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

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

Lon-Fye Lye, Natalia S. Akopyants, Deborah E. Dobson, Stephen M. Beverley

Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri, USA

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.

Received 29 May 2016 **Accepted** 15 June 2016 **Published** 4 August 2016

Citation Lye L-F, Akopyants NS, Dobson DE, Beverley SM. 2016. A *Narnavirus*-like element from the trypanosomatid protozoan parasite *Leptomonas seymouri*. Genome Announc 4(4):e00713-16. doi:10.1128/genomeA.00713-16.

Address correspondence to Stephen M. Beverley, beverley@wusm.wustl.edu.

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.

Provisionally, 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 mitochondrial 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.

ACKNOWLEDGMENTS

We thank members of our laboratory and Nicolas Fasel for discussions, as well as colleagues who provided *Leptomonas* strains that had been cultured independently.

This work was supported by grants NIH AID R56 AI099364 and RO1-AI29646 to S.M.B.

FUNDING INFORMATION

This work, including the efforts of Lon-Fye Lye, Natalia S. Akopyants, Deborah E. Dobson, and Stephen M. Beverley, was funded by HHS | National Institutes of Health (NIH) (R56 AI099364 and R01 AI 29646).

REFERENCES

1. **Wickner RB, Fujimura T, Esteban R.** 2013. Viruses and prions of *Saccharomyces cerevisiae*. Adv Virus Res **86:**1–36. http://dx.doi.org/10.1016/B978-0-12-394315-6.00001-5.
2. **Hillman BI, Cai G.** 2013. The family *Narnaviridae*: simplest of RNA viruses. Adv Virus Res **86:**149–176. http://dx.doi.org/10.1016/B978-0-12-394315-6.00006-4.

3. **Hillman B, Esteban R**. 2012. Family *Narnaviridae*, p 1055–1060. In King A, Adams M, Carstens E, Lefkowitz E (ed), *In* virus taxonomy: classification and nomenclature of viruses, 9th ed. Elsevier, San Diego, CA.
4. **Kraeva N, Butenko A, Hlaváčová J, Kostygov A, Myšková J, Grybchuk D, Leštinová T, Votýpka J, Volf P, Opperdoes F, Flegontov P, Lukeš J, Yurchenko V**. 2015. *Leptomonas seymouri*: adaptations to the dixenous life cycle analyzed by genome sequencing, transcriptome profiling and co-infection with *Leishmania donovani*. PLoS Pathog **11:**e1005127. http://dx.doi.org/10.1371/journal.ppat.1005127.
5. **Beiting DP, Peixoto L, Akopyants NS, Beverley SM, Wherry EJ, Christian DA, Hunter CA, Brodsky IE, Roos DS**. 2014. Differential induction of TLR3-dependent innate immune signaling by closely related parasite species. PLoS One **9:**e88398. http://dx.doi.org/10.1371/journal.pone.0088398.
6. **Fazeli CF, Habili N, Rezaian MA**. 1998. Efficient cloning of cDNA from grapevine leafroll-associated virus 4 and demonstration of probe specificity by the viral antibody. J Virol Methods **70:**201–211. http://dx.doi.org/10.1016/S0166-0934(97)00193-6.
7. **Rastgou M, Habibi MK, Izadpanah K, Masenga V, Milne RG, Wolf YI, Koonin EV, Turina M**. 2009. Molecular characterization of the plant virus genus *Ourmiavirus* and evidence of inter-kingdom reassortment of viral genome segments as its possible route of origin. J Gen Virol **90:** 2525–2535. http://dx.doi.org/10.1099/vir.0.013086-0.
8. **Simpson AG, Stevens JR, Lukes J**. 2006. The evolution and diversity of kinetoplastid flagellates. Trends Parasitol **22:**168–174. http://dx.doi.org/10.1016/j.pt.2006.02.006.
9. **Ghosh S, Banerjee P, Sarkar A, Datta S, Chatterjee M**. 2012. Coinfection of *Leptomonas seymouri* and *Leishmania donovani* in Indian leishmaniasis. J Clin Microbiol **50:**2774–2778. http://dx.doi.org/10.1128/JCM.00966-12.
10. **Ahuja K, Arora G, Khare P, Selvapandiyan A**. 2015. Selective elimination of *Leptomonas* from the *in vitro* co-culture with *Leishmania*. Parasitol Int **64:**1–5. http://dx.doi.org/10.1016/j.parint.2015.01.003.
11. **Ives A, Ronet C, Prevel F, Ruzzante G, Fuertes-Marraco S, Schutz F, Zangger H, Revaz-Breton M, Lye LF, Hickerson SM, Beverley SM, Acha-Orbea H, Launois P, Fasel N, Masina S**. 2011. *Leishmania* RNA virus controls the severity of mucocutaneous leishmaniasis. Science **331:** 775–778. http://dx.doi.org/10.1126/science.1199326.