

# **Complete RNA Virus Genomes Assembled from Murine Cecal Metatranscriptomes**

**Running title:** RNA Viral Genomes from Murine Metatranscriptomes

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## 1 **Abstract**

2 Efforts to catalogue viral diversity in the gut microbiome have largely ignored RNA viruses. To  
3 address this, we screened assemblies of previously published mouse gut metatranscriptomes  
4 for the presence of RNA viruses. We identified the complete genomes of a Astrovirus and 5  
5 Mitovirus-like viruses.

The viral fraction of the mammalian gut microbiome forms a crucial component in the relationship between microbe and host. Bacterial viruses serve as an important source of genetic diversity and population control for the microbiota, driving its ecology and evolution (1). Mammalian viruses disrupt the gut environment through infection and the response of the host immune system (2). Bacterial and mammalian viruses make significant contributions to host health and disease. Current efforts to describe the diversity of viruses present in the gut have focused on using shotgun metagenomics to identify double-stranded DNA viruses, predominantly bacteriophage and host pathogens [INSERT REFERENCE]. However, this method ignores viruses with RNA genomes, which make up a considerable portion of the environmental viromes (3).

We re-analyzed deeply-sequenced metatranscriptome data produced by our lab for the study of microbiome dynamics in a mouse model for *Clostridioides difficile* infection (4, 5). Briefly, C57Bl/6 mice from a breeding colony we maintain at the University of Michigan were treated with one of three different antibiotics (clindamycin, streptomycin, or cefoperazone). After a 24 hour recovery period, the mice were infected with *C. difficile* strain 630. Cecal contents were removed from each animal 18 hours post infection and frozen for RNA extraction and sequencing. RNA sequences from each sample were assembled individually using rnaSPAdes v3.13.1 (6) and concatenated for dereplication, resulting in **R 70,779** contigs longer than 1 kb. Contigs were then screened against a custom RefSeq database of viral RNA-dependent RNA polymerase (RdRP) protein sequences with a maximum e-value of  $10^{-20}$ , resulting in **R 22** contig hits. RdRP is conserved amongst almost all RNA viruses without a DNA stage in genome replication. These contigs were then annotated with Interproscan v5.39-77.0 (7, 8). We constructed phylogenetic trees from RdRP protein sequences using IQ-TREE v1.6.12 (9).

Two classes of RNA viruses were assembled with high coverage with sequences originating from most of the mouse treatment groups, including germ-free mice. First, a **R 6811** base-long astrovirus genome was obtained with **R 1683**-fold coverage (Figure 1A). The genome contained 3 predicted open reading frames encoding a capsid, RdRP, and a trypsin-like peptidase. Second, 5 distinct, but closely related RNA virus genomes ranging in length from **R 2309 to 2447** bases with **R XXXXX** to **R XXXXXX**-fold coverage belonged to a previously undescribed clade of Narnaviridae adjacent to the Mitoviruses (Figure 1B). These RNA virus genomes will facilitate future studies of

35 RNA virus biology in the murine microbiome.

36 **Data Availability.** The RNA-seq data are available the NCBI Sequence Read Archive  
37 (SRA) database under the accession numbers PRJNA354635 (*C. difficile* infected mice)  
38 and PRJNA415307 (mock-infected mice). The assembled genomes are available at the  
39 National Center for Biotechnology Information (NCBI) GenBank under the accession numbers  
40 MN780842-MN780847. All of the scripts and software used to perform this analysis are available  
41 at [https://github.com/JMAStough/Stough\\_Mouse\\_RNA\\_Virome\\_MRA\\_2019](https://github.com/JMAStough/Stough_Mouse_RNA_Virome_MRA_2019).

## 42 **Acknowledgements**

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70 **Figure 1. Phylogenetic trees showing the relatives of the metatranscriptome assembled**  
71 **genomes.** Maximum Likelihood phylogenetic trees constructed from RdRP amino acid sequences  
72 for (A) Astroviruses and (B) Narnaviruses. Node annotations represent IQTree Ultra-fast Bootstrap  
73 statistics, values less than 50% were excluded from the tree. Scale bars are marked in red to the  
74 left of each tree. Highlight colors in (B) represent major Narnavirus taxa: Orange - Ourmiaviruses,  
75 Pink - Ourmia-like Mycoviruses, Gray - Narnaviruses, Blue - Mitoviruses, Purple - Murine  
76 Mitovirus-like viruses, Green - Leviviruses.