



Draft Genome Sequences of Six Novel Picorna-Like Viruses from Washington State Spiders

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ABSTRACT We report draft genome sequences of six novel *Picornavirales* members from six different spider species found in Washington state. These six viral sequences distinctly clustered together phylogenetically with less than 35% amino acid identity to the closest reference viral genome.

As new arthropod-borne zoonoses become of increasing relevance to human health it has become clear that greater understanding of the arthropod virome is required (1). Spider metagenomics provides an ample area for viral discovery, as spiders both are undersequenced and act as arthropod aggregators that are windows into the greater invertebrate virosphere (2).

All spiders were sampled in western Washington and stored in ethanol for <1 year before sequencing. *Hexura picea* Simon is a native mygalomorph spider of the family *Mecicobothriidae*; *Metellina curtisi* (McCook) is a native orbweaver of the family *Tetragnathidae*; *Hyptiotes gertschi* Chamberlin & Ivie is a native triangle-weaving orbweaver. *Theridion simile* C. L. Koch is a native cobweb weaver of Holarctic distribution. *Theridion tinctum* (Walckenaer) is an introduced cobweb weaver and *Xysticus cristatus* (Clerck) is an introduced crab spider, both of European origin.

Metagenomic libraries were performed as described previously (3). Lysates from bead-beaten spiders were spin-filtered through a 0.45 μ M filter and RNA was extracted. Double-stranded cDNA was tagged using Nextera XT and amplified with 19 cycles of PCR (4). Adapter-trimmed reads were assembled SPAdes v3.8 and translated open reading frames (ORFs) >2 kb were aligned to PDB via HHPred (5, 6).

From the *Theridion simile* spider, an 8,825 nucleotide (nt) contig with one continuous open reading frame was assembled corresponding to the novel *Picornavirales* Rainier virus UW1. The translated open reading frame aligned 29% by amino acid to *Solenopsis invicta* virus-1 (NC_006559) (7). From the *Xysticus cristatus* spider, a 10,001 nucleotide contig containing a singular ORF was assembled, corresponding to Crab spider picornavirus. The translated ORF aligned 28% by amino acid to the Israeli acute paralysis virus (8).

From the *Hexura picea* spiders, two contigs of 9,798 and 10,025 nt were assembled with ORFs of 9,734 and 9,922 nt, respectively. The translated ORFs aligned 82% by amino acid, constituting two strains (BG1 and RC1) of the novel Burke-Gilman virus. They aligned 33% by amino acid to the polyprotein of the Rice tungro spherical virus, a member of the *Secoviridae* family (9). These two viruses also aligned 31 to 33% by amino acid to the Rainier virus UW1, Crab spider picornavirus, and Baker virus.

Partial genome sequences from three novel species from this likely novel clade were also recovered. Metagenomic sequences from a *Metellina curtisi* spider contained a 3,992 nt contig that aligned most closely to the aphid lethal paralysis virus with an

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amino acid identity of 24%. This contig corresponded to the structural protein of Volivirus B7-MC0. The *Hyptiotes gertschi* assembly contained a contig of 3,256 bases (Swagivirus HG) and aligned 35% by amino acid to the helicase of Aichi virus 1 (10). The *Theridion tinctum* assembly included a contig of 2,421 bases and aligned 47% by amino acid to the Aphis glycines virus 1 capsid polyprotein (KF360262).

These viruses likely form a new clade within the *Picornavirales* order as they cluster closer to each other than to any of the reference viral sequences listed here. Further taxonomic, sequencing, and functional studies would be required to fully delineate their place within the *Picornavirales* order.

Accession number(s). The sequences described in this study are deposited in DDBJ/ENA/Genbank under the accession numbers [KX779450](#) (Crab spider picornavirus J1), [KX782311](#) and [KX782312](#) (Burke-Gilman virus RC1 and BG1), [KX782313](#) (Rainier virus UW1), [KY316057](#) (Noveria virus B8-Tt), [KY316058](#) (Volivirus B7-MC0), and [KY316059](#) (Swagivirus HG B7-Hg).

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