Rare and diverse giant viruses unexpectedly found in a forest soil ecosystem

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Biologist Jeff Blanchard, kneeling at center in hat, collects soil samples surrounded by students at Harvard Forest in central Massachusetts. He and his Ph.D. student Lauren Alteio unexpectedly discovered large-genome giabnt viruses there. Credit: UMass Amherst

Until recently, scientists thought of viruses as mostly small infectious agents, tiny compared to typical bacteria and human cells. So imagine the surprise when biologist Jeff Blanchard and Ph.D. student Lauren

Alteio at the University of Massachusetts Amherst, with others at the U.S. Department of Energy's (DOE) Joint Genome Institute (JGI), discovered giant viruses—relatively speaking the size of Macy's parade day balloons—in soil at Harvard Forest in Petersham, Massachusetts.

"We were not looking for giant viruses," says Blanchard. "Our goal was to isolate bacteria directly from the environment to understand how microbial communities are changing in response to soil warming."

For this work, the researchers suspended microbial cells from the soil in a mild detergent solution, added a non-toxic DNA-binding dye, then used fluorescence-activated cell sorting (FACS) to isolate individual cells, Blanchard explains. Giant viruses, up to hundreds of times larger than other viruses, have extremely large genomes and are captured by this method because of their similarity in size to bacteria, he notes.

A collaborator and senior scientist at the JGI in Walnut Creek, California, Tanja Woyke, suggested they then use a new strategy, mini-metagenomics, for putting the cells into small pools before sequencing and assembling their genomes. This resulted in DNA sequences from over 2,000 individual cells and/or particles, Blanchard reports. In the pools they found 16 new giant viruses, which was "a wonderful surprise and very exciting new science," he adds.

Co-first author with Alteio of the paper in *Nature Communications*, JGI bioinformaticist Frederik Schulz, who helped Alteio to identify new soil bacteria and archaea in the mini-metagenomic data, says, "The fact that we found all these giant virus genomes in soil was especially intriguing, as most of the previously described giant viruses were discovered in aquatic habitats. The metagenomic data generated here from a single sampling site contained far more new giant virus genomes than any other data set I have seen to date."

Blanchard adds, "Our research is usually focused on the effects of soil warming, but this new mini-metagenomic approach has uncovered a trove of viral and bacterial biodiversity in species groups we don't typically associate with the soil. There are a number of mysteries we'll be following up on."

Schulz points out, "We recovered 16 distinct giant virus genomes in this study, but we are merely scratching the surface. If we sample more at the same site this number would easily double, triple or even quadruple." The authors say results illustrate that using new methods "can lead to key discoveries."

The giant virus discovery is related to long-term soil-warming experiments in place for many years at the research forest about 28 miles northeast of the UMass Amherst campus, where heating cables similar to those used to keep football and soccer fields from freezing are buried about 4 inches (10 cm) under several plots. The cables keep the soil surface 5 degrees Celsius warmer than the ambient temperature, creating an outdoor laboratory of artificial climate change, Blanchard says.

In most giant virus research, he says, researchers cultivate a protist or amoeba host which attracts viruses that usually infect it, a labor-intensive process. "They're hard to work with, and only viruses that grow in that host will be cultivated," he notes. "There are millions of potential host species and it would be impossible to use this approach with them all." By contrast, isolating cells directly from the environment and using mini-metagenomics methods yields genomic data at a lower cost, he says.

"Tanja is famous for sequencing genomes of hard-to-cultivate organisms from environmental samples and she had the intuition that if we took this new approach, new branches of life would be revealed," Blanchard says. "While using this method we don't know what our giant viruses look like; one could try to repeat the experiment in future research to image some particles after they are sorted."

The UMass Amherst microbiologist adds, "Not only did we just discover many new giant viruses, but we did it using a thimbleful of soil. It would be nice to characterize these viruses one at a time, there's a lot of skill and art in that. But it would be a years-long project. Finding 16 at once is kind of overwhelming, and none of them are the same. If you think of all the soil in the world, if there are 10,000 species of bacteria in a gram of soil, about a teaspoon, imagine how many new giant viruses are out there."

Woyke adds, "To me, the most intriguing and eye-opening part of the study was the high number and diversity of major capsid proteins, which is like a barcode for giant viruses, found in the unassembled bulk soil metagenome. Deep sequencing of soil metagenomes is revolutionizing our understanding of this very important terrestrial ecosystems with many exciting soil microbiome initiatives ongoing, yet our data emphasizes that still many missing pieces to the puzzle remain."

The scientists gave the new species names that reflect their forest origins, such as "Dasovirus" Greek "daso" for forest and "Solumvirus" for Latin "solum" soil. They also propose naming one "Harvovirus" to honor Harvard Forest.

More information: Frederik Schulz et al, Hidden diversity of soil giant viruses, *Nature Communications* (2018). DOI: 10.1038/s41467-018-07335-2

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