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Searching for Megaviruses in Iceland

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Presence of Megaviruses from Diverse Icelandic Environments

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ABSTRACT

The proposed Megavirales order comprises members of the previously known nucleocytoplasmic large DNA viruses (NCLDVs). Virus families in the Megavirales order include Poxviridae, Ascoviridae, and the recently explored families of megaviruses infecting free living amoeba such as Mimiviridae, Marseilleviridae, and Pandoraviridae. Megaviruses have been isolated from water and soil samples from Chile, France, India, and the United States. We chose to study the occurrence of megaviruses in Iceland because of the diverse habitats all within one island. No research has been carried out on the presence of megaviruses in Iceland. Samples of water and soil were collected from lava fields, moss/lichen mountain fields and mixed forestry plantations. *Acanthamoeba castellanii* (ATCC #30010) at a 5×10^5 cells/ml concentration was used in an amoebal co-culture. A 1:10 dilution of soil or water sample preparation was added to each well. Cell confluence estimates were taken immediately after inoculation and plates were incubated at 28°C. After 72 hours, wells containing mixed forestry plantation soil saw 48% lysis, followed by 44% lysis in wells containing lava field soil, and 59% lysis in wells containing moss/lichen mountain field soil. The co-cultures were subcultured onto *A. castellanii* plus antibiotics at a 1:10 dilution. Percent lysis for wells containing mixed forestry plantation soil, lava field soil, and moss/lichen mountain field soil was 19%, 22%, and 31% respectively. These data suggest *A. castellanii* may be infected with viruses, potentially megaviruses, originating from the various samples.

BACKGROUND

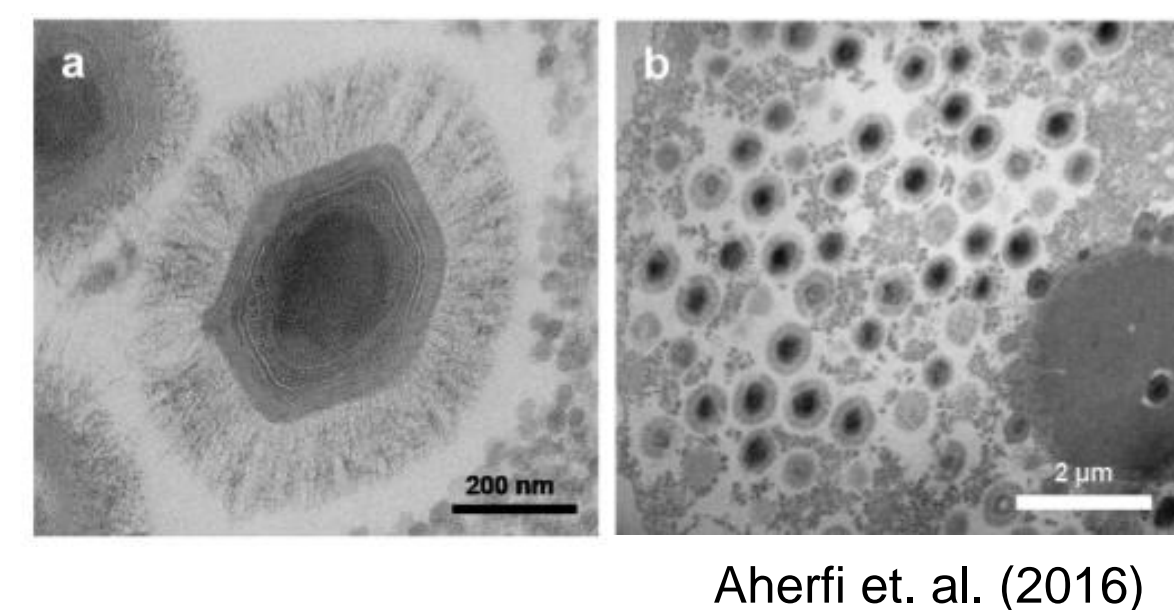


Figure 1. Electron micrographs of (a) Mimivirus and (b) Mimivirus viral factories (Aherfi et. al (2016)). Megavirales viruses have a diameter 150-750nm and genome lengths of 17-1.25Mb. Relative to other viruses, they replicate rather independently of the host cell in viral factories next to the nucleus as seen in (b) and encode histone-like proteins and translational machinery such as amino-acyl tRNA synthetases.



Figure 2. A 2016 map of locations in which Megaviruses have been found. Megaviruses have been found in natural and manmade environments around the globe. No research has been done on their presence in Iceland (black arrow).

SAMPLING LOCATION



Figure 3. Iceland was chosen as a sampling location due to the wide variety of soil types and water sources on a relatively small area of land. Samples were collected from lava field soils, heath soils, glacial fed rivers, man-made forests, black sand beaches, and brackish waters.

SAMPLING

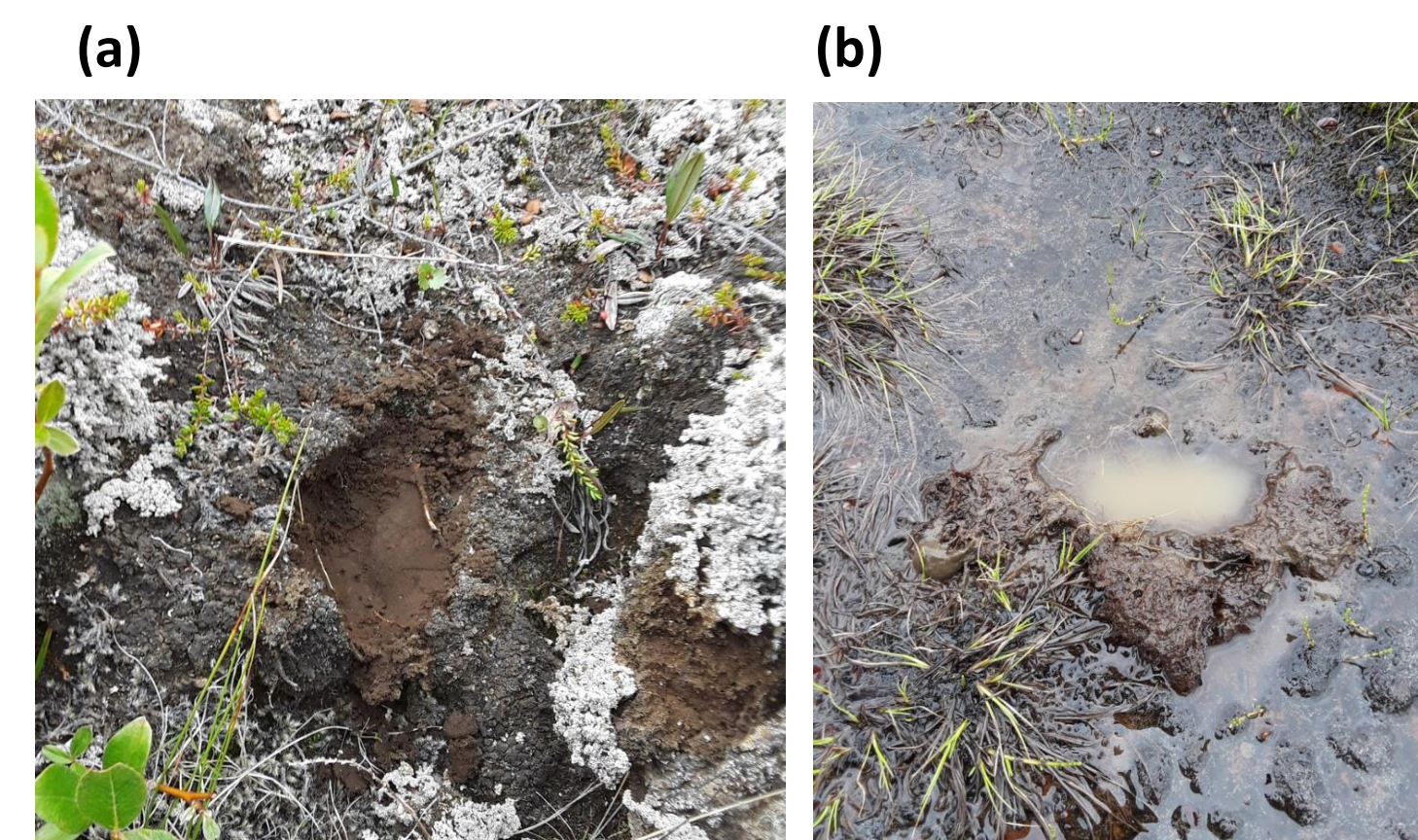


Figure 4. The top 4cm of soil was collected with a sterile scoop. 2-3 replicates were taken on a 5m transect. Soil was held at 4 °C until time of analysis. (a) Icelandic lava field shrub heaths, (b) moss and lichen mountain fields

METHODS

ACANTHAMOEBA CASTELLANII CO-CULTURES

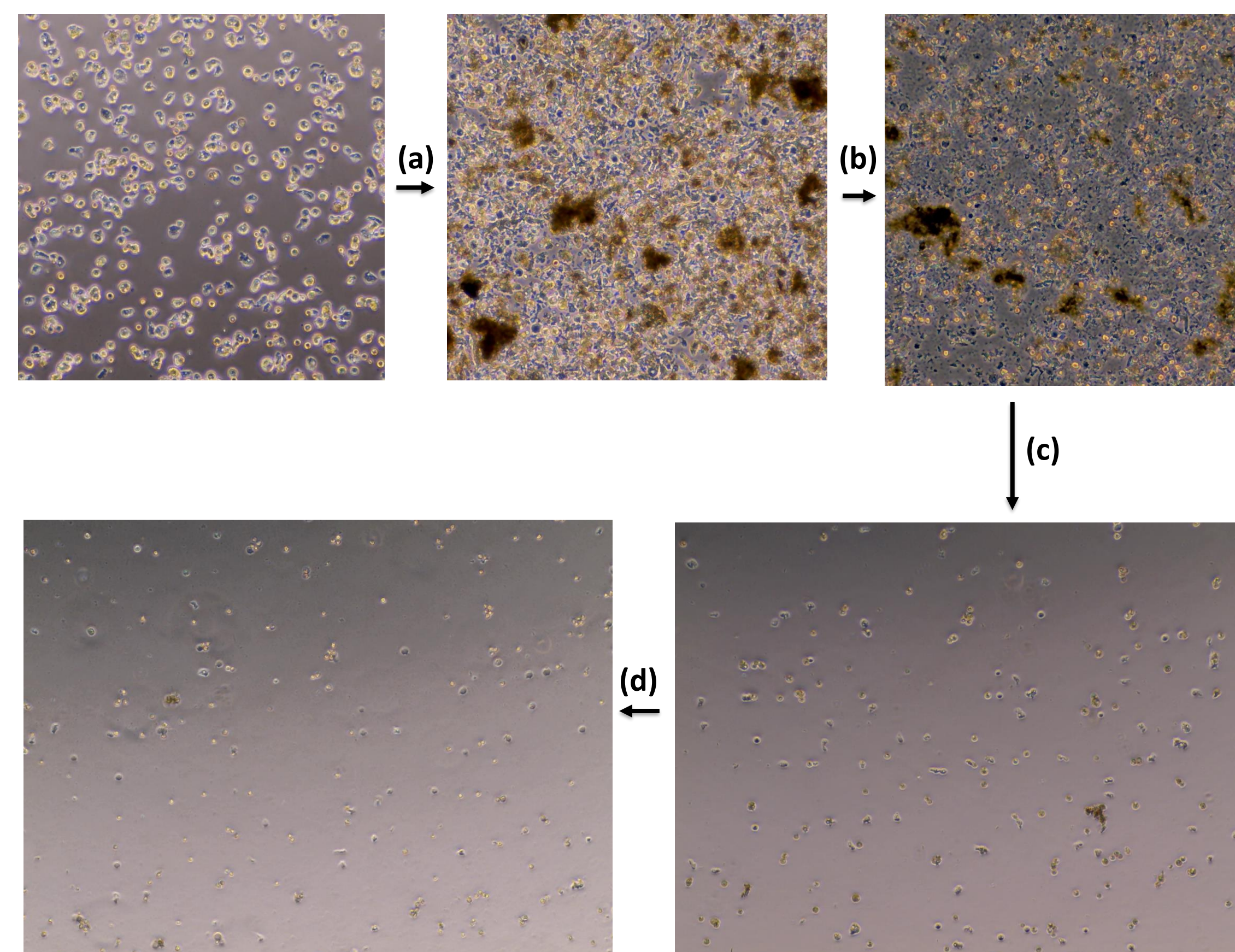


Figure 5. Co-culture flow chart
(a) A 1:10 dilution of soil or water was added to a fresh culture of *Acanthamoeba castellanii* (5×10^5 cells/ml).
(b) Inoculated cultures were incubated at 28°C for 3-5 days and were observed for lysis.
(c) Wells exhibiting lysis were sub-cultured onto a fresh amoebal culture containing an antibiotic cocktail (4 µg/mL ciprofloxacin, vancomycin, and rifampicin, 500 IU/mL colimycin, and 100 µg/mL Amphotericin B) to exclude amoeba lysing bacteria and fungi.
(d) Plates were incubated at 28°C for 3-5 days and were observed for lysis.

PHASE GENOMICS METAGENOMIC ANALYSIS

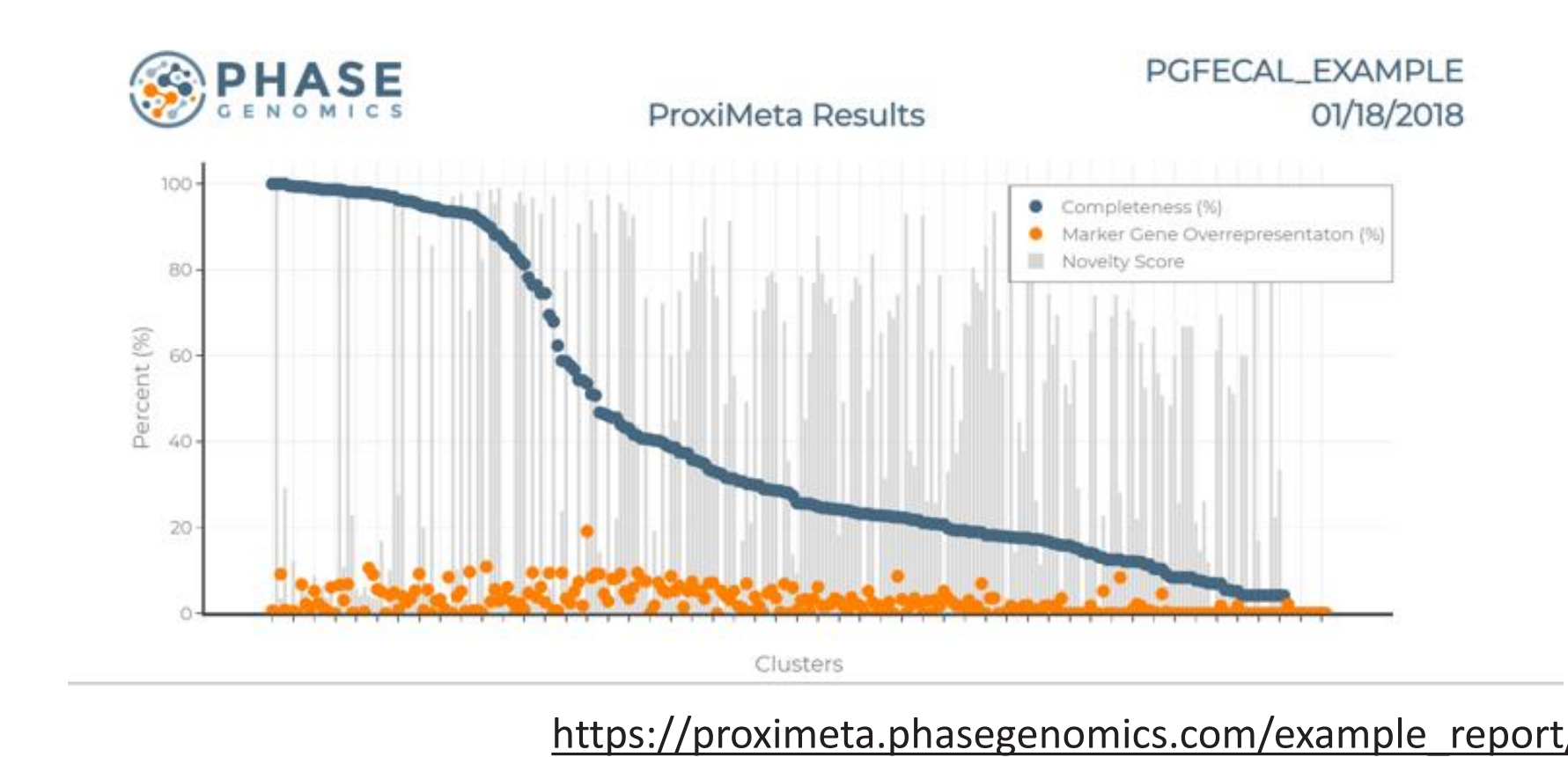


Figure 6. The moss and lichen field and mixed forestry plantation samples were sent to Phase Genomics for metagenomic sequencing.

QUESTION

1. Are megaviruses present in Iceland?

RESULTS

Percent lysis of amoeba in various Icelandic soils

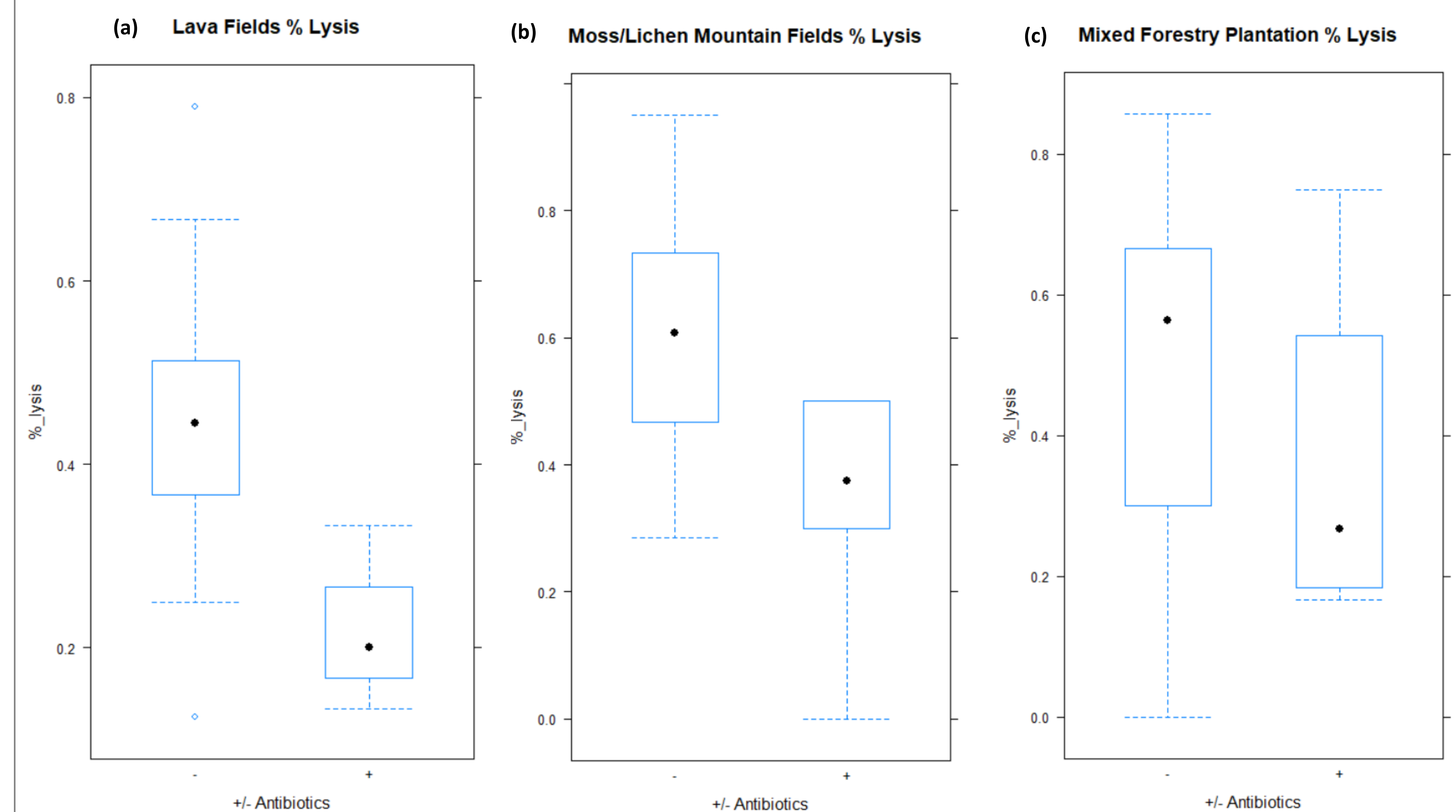


Figure 7. Percent lysis of *A. castellanii* co-cultures was taken by visual estimation of cell confluence at 3-5 days post-inoculation compared to 0 hours post-inoculation. R-studio was used to generate box plots showing percent lysis from three samples treated with (+) and without (-) an antibiotic cocktail. In each figure, a reduction in lysis is seen in co-cultures treated with antibiotics. (a) In amoebal cultures inoculated with soil from Icelandic lava field shrub heaths, a range of 13-33% lysis with a 20% median is seen for cultures treated with antibiotics, while a range of 12-78% lysis with a median of 44% is seen for untreated cultures. (b) In amoebal cultures inoculated with soil from moss and lichen mountain fields, a range of 0-50% lysis with a 37% median is seen for cultures treated with antibiotics, while a range of 28-95% lysis with a median of 61% is seen for untreated cultures. (c) In amoebal cultures inoculated with soil from a mixed forestry plantation, a range of 16-75% lysis with a median of 27% is seen for cultures treated with antibiotics while a range of 0-85% lysis with a 56% median is seen for untreated cultures.

CONCLUSIONS

- Amoeba lysing microorganisms exist in Icelandic soils
- Non-bacterial and non-fungal amoeba lysing microorganisms exist in Icelandic soils

FUTURE DIRECTIONS

- PCR on soil samples using Polymerase B primers for 7 different *Megaviridae* species to confirm megavirales presence.
- Phase Genomics Metagenomic analysis to determine soil microbiome and megavirus presence

REFERENCES CITED

- Aherfi S, Colson P, La Scola B, Raoult D. 2016. Giant viruses of amoebas: An update. *Frontiers in Microbiology* 7:349.

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