NOVEL VIRUSES DISCOVERED IN *PHYTOPHTHORA HEVEAE* AND TWO NOVEL *PHYTOPHTHORA* CLADE 5 SPECIES FROM PANAMA

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Introduction

Over the past years, an increasing number of novel viral species have been discovered in many major phytopathogenic fungal taxa, as well as in some oomycetes. Their genomes are mainly organized in double-stranded RNA (dsRNA). However, many recent studies have reported viruses with positive-sense single-stranded RNA (+ssRNA) and negative-sense single-stranded (-ssRNA) genomes in fungi and in oomycetes, as well as a few single-stranded DNA (ssDNA) viruses that have been characterized from fungal pathogens [4]. *Phytophthora* is a genus of oomycetes that includes some of the most devastating plant pathogens with a broad host range including important crop and tree species [3]. *Phytophthora* Clade 5 is one of the smallest clades of this genus currently consisting of four species: *P. agathidicida*, *P. castaneae*, *P. cocois* and *P. heveae* [7]. Viruses of oomycetes, including viruses of *Phytophthora* spp., have been understudied compared to fungal viruses, but with the development of novel technologies such as High-Throughput Sequencing (HTS), the number of viruses detected in these organisms is increasing [1, 2, 6]. Discovery of novel viral species residing in fungal and oomycete hosts could help in understanding the evolution and ecology of viruses in general, as these viruses are often related to viruses infecting other organisms. Further study of this virus group may also lead to designing biological control methods for suppression of their phytopathogenic hosts.

In this study, a combination of traditional and novel virus detection methods was used to investigate the potential virome of *P. heveae* and two new species belonging to *Phytophthora* Clade 5, informally designated as *P. sp. castaneae-like* and *P. sp. myristicae_castaneae-like*. Isolates of all three species used in this study were isolated from a tropical lowland rainforest and tropical hill forest in Panama.

Materials & Methods

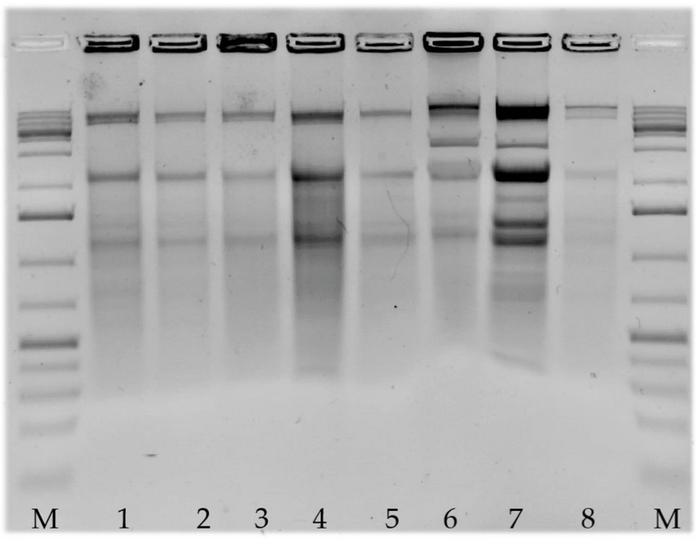
A total of eight isolates from three taxa (*P. heveae* (isolates PA50, 51 and PA53) and two novel Clade 5 species, informally designated as *Phytophthora sp. castaneae-like* (isolate PA190) and *Phytophthora sp. myristicae castaneae-like* (isolates PA290, 298, 299 and 300)) were screened for viruses using a modified dsRNA extraction protocol based on CF-11 cellulose chromatography [5].

Necrotic lesions caused by *Phytopthora* species (photo: Y. Balci).

After observing various banding patterns indicating putative viral presence, the total RNA of these isolates was extracted, pooled together and sent for library preparation and sequencing by HTS of total stranded RNA to SEQme s.r.o., Czech Republic. Bioinformatics analyses of RNA seq reads consisted of de-novo assembly by Trinity (version 2.6.5) and BLASTX (version blast+ 2.10.0) comparison.

Results

The dsRNA extraction resulted in multiple banding patterns in each of the eight isolates. According to the BLASTX results, the putative viruses resemble members of the order *Bunyavirales* (-ssRNA) and virus families *Chrysoviridae* (dsRNA), *Endornaviridae* (+ssRNA), *Megabirnaviridae* (dsRNA), *Narnaviridae* (+ssRNA), *Totiviridae* (dsRNA), the proposed families “Fusagraviridae” (dsRNA), “Fusariviridae” (+ssRNA) and, the virus group provisionally named “Ustiviruses” (dsRNA). The presence of the viral contigs is going to be validated by RT-PCR.



dsRNA banding patterns of screened isolates followed by agarose gel electrophoresis. Lane 1: PA50; lane 2: PA51; lane 3: PA53; lane 4: PA190; lane 5: PA290; lane 6: PA298; lane 7: PA299; lane 8: PA300. M is DNA marker (GeneRuler 1 kb Plus DNA Ladder, 75–20,000 bp, Thermo Scientific, MA, USA)

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