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Viral diversity in *Phytophthora cactorum* population infecting strawberry

Anna Poimala,[1*] **Päivi Parikka**,[2] **Jarkko Hantula**[1] **and Eeva J. Vainio**[1]

[1]*Natural Resources Institute Finland (Luke), Forest Health and Biodiversity, Latokartanonkaari 9, Helsinki, FI-00790, Finland.*

[2]*Natural Resources Institute Finland (Luke), Plant Health, Humppilantie 18, Jokioinen, 31600, Finland.*

Summary

Eighty-eight *Phytophthora cactorum* strains isolated from crown or leather rot of strawberry in 1971–2019 were screened for viruses using RNA-seq and RT-PCR. Remarkably, all but one isolate were virus-infected, most of them harbouring more than one virus of different genera or species. The most common virus occurring in 94% of the isolates was the Phytophthora cactorum RNA virus 1 (PcRV1) resembling members of *Totiviridae*. Novel viruses related to members of *Endornaviridae*, named Phytophthora cactorum alphaendornaviruses 1-3 (PcAEV1-3), were found in 57% of the isolates. Four isolates hosted viruses with affinities to *Bunyaviridae*, named Phytophthora cactorum bunyaviruses 1-3 (PcBV1-3), and a virus resembling members of the proposed genus 'Ustivirus', named Phytophthora cactorum usti-like virus (PcUV1), was found in a single isolate. Most of the virus species were represented by several distinct strains sharing ≥81.4% aa sequence identity. We found no evidence of spatial differentiation but some temporal changes in the *P. cactorum* virus community were observed. Some isolates harboured two or more closely related strains of the same virus (PcAEV1 or PcRV1) sharing 86.6%–96.4% nt identity in their polymerase sequence. This was surprising as viruses with such a high similarity are typically mutually exclusive.

Introduction

The Oomycete genus *Phytophthora* comprises of many plant pathogens that cause significant economic losses in agricultural, silvicultural and natural ecosystems all over the world (Erwin and Ribeiro, 1996; Jung *et al*., 2018). *Phytophthora* species, as many other oomycete genera, share a similar lifestyle with fungi but are more closely related to brown algae and belong to the Kingdom Stramenopila (Heterokonta). *Phytophthora cactorum* is an omnivorous pathogen that can infect a wide variety of plant species (Erwin and Ribeiro, 1996). On cultivated strawberry, it is the causative agent of strawberry crown rot as well as strawberry leather rot of fruits. Both diseases are reported to cause economic losses in strawberry production globally (Erwin and Ribeiro, 1996; Nellist *et al*., 2019). *Phytophthora cactorum* can persist in the soil as sexual oospores, which germinate under wet conditions to produce mycelium and sporangia, which release motile asexual zoospores to infect the plant collar and fruits (Chepsergon *et al*., 2020). The infection becomes visible as brown discoloration inside the collar, and subsequent wilting eventually results in the death of the plant. The degree of resistance to the disease varies among strawberry cultivars (Stensvand *et al*., 1999). These diseases are controlled mainly by selecting healthy plants for planting and by planting strawberries on ridges to improve drainage and aeration (hilling) but also sanitation and fungicides are used (Eikemo *et al*., 2003a).

Viruses infecting *Phytophthora* species have been discovered relatively recently, and only a few have been described. An alphaendornavirus named Phytophthora endornavirus 1 (PEV1) was described by Hacker *et al*. (2005) from *Phytophthora* taxon douglas fir in the USA, and later also found in *P. ramorum* isolates from several hosts in Europe (Kozlakidis *et al*., 2010). The family *Endornaviridae* includes viruses with linear single-stranded, positive-sense RNA genomes that range from 9.7 to 17.6 kb and contain one large open reading frame (ORF) coding for a polyprotein (Valverde *et al*., 2019). The two genera of *Endornaviridae*, *Alphaendornavirus* and *Betaendornavirus*, are separated based on genome size, host and the presence of unique genome domains. In addition to the PEV1, the causal agent of potato late blight, *P. infestans*, has been shown to host four dsRNA viruses named PiRV1-4 (Cai *et al*., 2009, 2012, 2013, 2018). Two of them, PiRV1 and PiRV2, represent

Received 24 January, 2021; revised 18 March, 2021; accepted 8 April, 2021. *For correspondence. E-mail anna.poimala@luke.fi; Tel. +358295322173.

putatively novel virus families, while PiRV4 resembles members of the family *Narnaviridae* and PiRV3 is affiliated with the proposed family 'Fusagraviridae' (Lee *et al*., 2017). PiRV2 was shown to stimulate the sporangia production of *P. infestans* thereby enhancing its virulence (Cai *et al*., 2019). Furthermore, a novel toti-like virus Phytophthora cactorum RNA virus 1 (PcRV1) was recently described in a *P. cactorum* isolate from a trunk lesion on silver birch (Poimala and Vainio, 2020). The family *Totiviridae* includes dsRNA viruses with monopartite linear genomes assigned to five genera; *Giardiavirus*, *Leishmaniavirus*, *Totivirus*, *Trichomonasvirus* and *Victorivirus* (King *et al*., 2011). However, several unclassified viruses resembling *Giardia lamblia* virus (GLV), the sole classified member of genus *Giardiavirus* (ICTV Master Species List 2018b. v2 at https://talk.ictvonline.org/), have been detected in recent years from arthropods, fish, fungi and oomycetes (Liu *et al*., 2012; Sasai *et al*., 2018; de Lima *et al*., 2019), among them also PcRV1 (Poimala and Vainio, 2020).

Viruses have also been found in association with other oomycetes. Novel (+)ssRNA viruses of phylum *Lenarviricota* (with affinities to *Narnaviridae*, *Botourmiaviridae* and *Leviviridae*) in addition to *Virgaviridae*-like, *Tobamovirus*-like and *Alphavirus*-like viruses, as well as novel (-)ssRNA viruses of orders *Bunyavirales*, *Mononegavirales*, *Serpentovirales* and viruses related to genus *Yuevirus* have been reported from *Plasmopara viticola* using metatranscriptomic approach (Chiapello *et al*., 2020). An unclassified Noda/Tombus-like (+)ssRNA virus has also been found in *P. halstedii* and *Sclerophthora macrospora* (Grasse and Spring, 2017). Novel dsRNA viruses found by Chiapello *et al*. (2020) included polymyco-, curvula-, partiti- and partiti-like viruses as well as toti- and toti-like viruses, botybirnaviruses, and an unclassified dsRNA virus. Viruses described in *Pythium* species include an unclassified gammapartitivirus (Shiba *et al*., 2018) as well as unclassified viruses showing resemblance to the GLV-like group of *Totiviridae* and the order *Bunyavirales* (Sasai *et al*., 2018; Shiba *et al*., 2019). Recently, eight putative virus species with genomic affinities to members of the order *Bunyavirales* were described in *Halophytophthora* (Botella *et al*., 2020). The order *Bunyavirales* includes viruses with linear, single-stranded, negative-sense or ambisense RNA genomes classified into 11 families (Abudurexiti *et al*., 2019), whose genomes generally comprise three unique molecules designated L (large), M (medium) and S (small), with sizes of 11–19 kb. The large segment generally codes for an RNA-dependent RNA polymerase (RdRp), and the M and S segments include genes for two external glycoproteins and a nucleocapsid respectively. In the case of most fungal bunyaviruses, only one genome segment (L) could be detected based on homology searches, but some are also found to have two to three genome segments (Lin *et al*., 2019; Nerva *et al*., 2019; Velasco *et al*., 2019).

In this study, we aimed to characterize the virus community of *P. cactorum* infecting strawberry plants in Finland and to assess the abundance, genetic variability as well as spatial and temporal occurrence of the viruses.

Results

Viral species, strains and sequence variants found in Phytophthora cactorum

The screening for dsRNA by cellulose affinity chromatography resulted in the detection of multiple viral RNA elements in most of the isolates (Fig. 1). Four dsRNA elements with sizes of circa 13 kb (1), 6 kb (2), 3,4 kb (3) and 2,4 kb (4) were present. The banding patterns in the 45 screened isolates are listed in Table 1. The only isolate with no visible dsRNA was PhF66.

The RNA-seq pools each yielded 122–165 M paired reads of 100 bp (data available in GenBank under BioProject PRJNA686859). Raw reads from each of the RNA pools were *de novo* assembled to obtain putative contiguous sequences (contigs) and subjected to BlastX for virus identification. The contigs were then used for primer design to be able to identify the isolates hosting the viruses using RT-PCR. In order to eliminate redundant contigs, to estimate the level of intragroup sequence variation and to investigate the possible occurrence of *in silico* chimeric contigs, all the Trinity contigs representing each viral genus or species were assembled in Geneious R10.

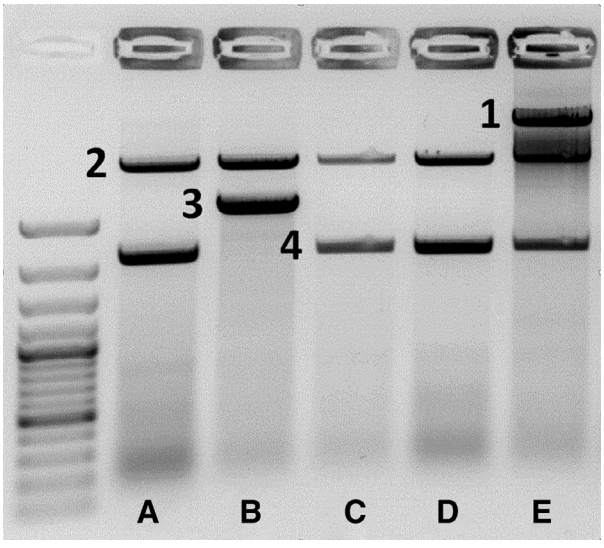

Fig 1. Examples of dsRNA elements detected in the *Phytophthora cactorum* isolates by cellulose affinity chromatography followed by agarose gel electrophoresis. DsRNA from isolates: (A) PhF88, (B) PhF79, (C) PhF90, (D) PhF103, (E) PhF115. The DNA ladder is GeneRuler 100 bp Plus DNA Ladder (Thermo Scientific).

Table 1. The 88 *Phytophthora cactorum* isolates of this study and their origins.

Isolate name	RNA-seq pool or screening method	CF11 banding pattern	Viruses	Isolation year	Country of origin	Strawberry cultivar	Disease	County
PhF5	RT-PCR	n.d.	PcRV1	1990	FIN	Jonsok	Crown rot	Pohja
PhF8	Phyto01	1,2,4	PcAEV1, PcAEV2, PcRV1	1990	FIN	Jonsok	Crown rot	Pohja
PhF9	Phyto01	1,2,4	PcAEV1, PcAEV2, PcRV1	1990	FIN	Jonsok	Crown rot	Pohja
PhF18	RT-PCR	n.d.	PcRV1		FIN		Crown rot	Piikkiö
PhF79	Phyto01	2,3	PcBV1, PcBV2, PcBV3, PcRV1, PcUV1	1991	FIN	Jonsok	Crown rot	Pohja
PhF23	RT-PCR	n.d.	PcAEV3, PcRV1	1992	FIN		Crown rot	Artjärvi
PhF101	Phyto01	1,2,4	PcAEV1, PcAEV3, PcRV1	1993	FIN	Jonsok	Crown rot	Pohja
PhF33	Phyto01	1,2,4	PcAEV1, PcAEV2, PcRV1	1993	FIN		Crown rot	
PhF38	Phyto01	1,2,4	PcAEV1, PcAEV2, PcRV1	1993	FIN		Crown rot	Ilomantsi
PhF44	RT-PCR	n.d.	PcRV1		FIN		Crown rot	Suonenjoki
PhF107	Phyto04	1,2,4	PcAEV3, PcRV1	1997	FIN		Crown rot	
PhF89	Phyto04	2,4	PcRV1		FIN		Leather rot (berry)	Suonenjoki
PhF90	Phyto01	2,4	PcRV1	1997	FIN		Crown rot	
PhF92	RT-PCR	1,2,4	PcAEV1, PcAEV3	1997	FIN		Crown rot	
PhF103	Phyto04	2,4	PcRV1		FIN	Bolero	Crown rot	Piikkiö
PhF108	RT-PCR	2,4	PcAEV1, PcRV1	1999	FIN		Crown rot	
PhF109	Phyto04	2,4	PcRV1	1999	FIN		Crown rot	Vimpeli
PhF110	Phyto04	2,4	PcRV1	1999	FIN		Crown rot	
PhF111	RT-PCR	n.d.	PcAEV3, PcRV1	1999	FIN		Crown rot	Kangasniemi
PhF112	Phyto04	2,4	PcRV1	1999	FIN	Polka	Crown rot	Suonenjoki
PhF113	RT-PCR	1,2	PcAEV1, PcAEV3, PcRV1	1999	FIN		Crown rot	Northern Ostrobothnia
PhF114	RT-PCR	1,2,4	PcAEV1, PcAEV3, PcRV1	1999	FIN	Cornwallis	Crown rot	Piikkiö
PhF115	RT-PCR	1,2,4	PcAEV1, PcAEV3, PcRV1	1999	FIN	Polka	Crown rot	Piikkiö
PhF116	RT-PCR	1,2,4	PcAEV1, PcAEV3, PcRV1	2000	FIN	Cavendish	Crown rot	Piikkiö
PhF118	Phyto04	2,4	PcRV1	2000	FIN	Polka	Crown rot	
PhF119	RT-PCR	1,2,4	PcAEV1, PcAEV3, PcRV1	2000	FIN		Crown rot	
PhF125	Phyto04	2,4	PcRV1	2000	FIN	Elsanta	Crown rot	Suonenjoki
PhF126	Phyto05	1,2,4	PcAEV3, PcRV1	2004	FIN		Crown rot	Northern Ostrobothnia
PhF128	Phyto05	2,4	PcRV1	2004	FIN	Polka	Crown rot	Piikkiö
PhF129	Phyto05	2,4	PcRV1	2005	FIN	Polka	Crown rot	

(Continues)

Table 1. Continued

Isolate name	RNA-seq pool or screening method	CF11 banding pattern	Viruses	Isolation year	Country of origin	Strawberry cultivar	Disease	County
PhF66	Phyto01	no bands	PcBV1, PcBV2	2006	FIN	Polka	Crown rot	Nurmijärvi
PhF124	RT-PCR	n.d.	PcRV1	2006	FIN		Crown rot	Suonenjoki
PhF130	Phyto05	2,4	PcRV1	2006	FIN		Crown rot	
PhF2017/01	Phyto01	1,2,4	PcAEV1, PcAEV2, PcAEV3, PcRV1	2017	FIN		Crown rot	Suonenjoki
PhF2017/02	RT-PCR	n.d.	PcAEV1, PcAEV2, PcAEV3, PcRV1	2017	FIN	Wendy	Leather rot (berry)	Suonenjoki
PhF2017/03	Phyto02	1,2,4	PcAEV1, PcBV1, PcRV1	2017	FIN	Malwina	Crown rot	Suonenjoki
PhF07/18	RT-PCR	n.d.	PcAEV1, PcAEV2	2018	FIN	Renaissance	Crown rot	Suonenjoki
PhF09/18	Phyto05	2,4	PcRV1	2018	FIN	Polka	Leather rot (berry)	Asikkala
PhF11/18	RT-PCR	1,2,4	PcAEV1, PcAEV2, PcAEV3, PcRV1	2018	FIN	Honeoye	Crown rot	Suonenjoki
PhF12/18	Phyto02	1,2,4	PcAEV1, PcAEV2, PcAEV3, PcRV1	2018	FIN	Malling Centenary	Crown rot	Suonenjoki
PhF13/18	RT-PCR	1,2,4	PcAEV1, PcAEV2, PcAEV3, PcRV1	2018	FIN	Jonsok	Crown rot	Kuopio
PhF14/18	RT-PCR	1,2,4	PcAEV1, PcAEV2, PcAEV3, PcRV1	2018	FIN	Polka	Crown rot	Kuopio
PhF16/18	Phyto05	1,2,4	PcAEV1, PcAEV2, PcAEV3, PcRV1	2018	FIN	Polka	Crown rot	Suonenjoki
PhF19/18	Phyto05	2,4	PcRV1	2018	FIN	Polka	Crown rot	Kesälahti
PhF21/18	Phyto05	n.d.	PcRV1	2018	FIN	Sonata	Crown rot	Kesälahti
PhF23/18	Phyto02	1,2,4	PcAEV1, PcAEV3, PcRV1	2018	FIN	Honeoye	Crown rot	Kesälahti
PhF25/18	RT-PCR	n.d.	PcAEV1, PcAEV2, PcAEV3	2018	FIN	Polka	Crown rot	Suonenjoki
PhF1010/18	Phyto05	2,4	PcRV1	2018	FIN	Polka	Crown rot	Kokemäki
PhF1011/18	Phyto05	2,4	PcRV1	2018	FIN	Sonata	Crown rot	Kokemäki
PhF1013/18	Phyto05	n.d.	PcRV1	2018	FIN	Polka	Crown rot	Perniö
PhF01/19	Phyto03	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF02/19	Phyto03	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF03/19	Phyto03	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF04/19	Phyto03	n.d.	PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF05/19	Phyto03	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF06/19	Phyto03	n.d.	PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF07/19	Phyto03	n.d.	PcRV1	2019	FIN	Honeoye	Crown rot	Suonenjoki
PhF08/19	Phyto03	n.d.	-	2019	FIN	Polka	Crown rot	Suonenjoki
PhF09/19	Phyto03	n.d.	PcRV1	2019	FIN	Wendy	Crown rot	Suonenjoki
PhF10/19	Phyto03	n.d.	PcRV1	2019	FIN	Wendy	Crown rot	Suonenjoki

(Continues)

Table 1. Continued

Isolate name	RNA-seq pool or screening method	CF11 banding pattern	Viruses	Isolation year	Country of origin	Strawberry cultivar	Disease	County
PhF11/19	Phyto03	n.d.	PcRV1	2019	FIN	Wendy	Crown rot	Suonenjoki
PhF12/19	Phyto03	n.d.	PcRV1	2019	FIN	Wendy	Crown rot	Suonenjoki
PhF13/19	Phyto03	n.d.	PcRV1	2019	FIN	Wendy	Crown rot	Suonenjoki
PhF14/19	Phyto03	n.d.	PcRV1	2019	FIN	Wendy	Crown rot	Suonenjoki
PhF15/19	Phyto03	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Sotkamo
PhF16/19	Phyto03	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Sotkamo
PhF17/19	Phyto03	n.d.	PcRV1	2019	FIN	Polka	Crown rot	Huhdasjärvi
PhF18/19	Phyto03	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Magnum	Crown rot	Suonenjoki
PhF19/19	Phyto03	n.d.	PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF20/19	Phyto03	n.d.	PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF21/19	Phyto03	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Sonata	Crown rot	Suonenjoki
PhF22/19	Phyto03	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Vesanto
PhF23/19	Phyto04	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Laitila
PhF24/19	Phyto04	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF25/19	Phyto04	n.d.	PcAEV1, PcAEV2, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF26/19	Phyto04	n.d.	PcAEV1, PcAEV2, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF27/19	Phyto04	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Magnum	Crown rot	Suonenjoki
PhF28/19	Phyto04	n.d.	PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF29/19	Phyto04	n.d.	PcAEV1, PcAEV2, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Suonenjoki
PhF30/19	Phyto04	n.d.	PcAEV1, PcRV1	2019	FIN	Magnum	Crown rot	Suonenjoki
PhF31/19	Phyto04	n.d.	PcAEV1, PcAEV3, PcRV1	2019	FIN	Polka	Crown rot	Oravisalo
PhF32/19	Phyto04	n.d.	PcRV1	2019	FIN		Crown rot	Sauvo
PhF33/19	Phyto04	n.d.	PcAEV1, PcAEV2, PcAEV3, PcRV1	2019	FIN	Flair	Crown rot	Suonenjoki
PhF34/19	Phyto04	n.d.	PcAEV1, PcAEV2, PcAEV3, PcRV1	2019	FIN		Crown rot	Suonenjoki
PhF35/19	Phyto04	n.d.	PcRV1	2019	FIN	Polka	Crown rot	Rautalampi
BBA62642	Phyto02	1,2,4	PcAEV1, PcAEV2, PcAEV3	1971	GER			
NY422	Phyto02	1,2,4	PcAEV1, PcAEV2, PcBV2, PcRV1	1990s	USA		Crown rot	
NY577	Phyto01	1,2,4	PcAEV1, PcAEV3, PcRV1	1990s	USA		Leather rot (berry)	

All isolates are from either crown rot or leather rot of strawberry (*Fragaria* × *ananassa*). The screening method (RNA-seq or RT-PCR), as well as the dsRNA banding pattern in CF11 cellulose affinity chromatography analyses, are shown, if applicable. n.d. = not determined.

The identified viruses included three positive-sense ssRNA viruses related to members of the genus *Alphaendornavirus*, three negative-sense ssRNA viruses related to members of the order *Bunyavirales* and one dsRNA virus affiliated with unclassified viruses provisionally named 'ustiviruses' (Herrero, 2016). Moreover, we found sequences of the toti-like dsRNA virus PcRV1 in numerous host isolates (Poimala and Vainio, 2020) (Tables 2–4). The highly common dsRNA element of ca. 2 kb (element 4 in Fig. 1) was cloned and subjected to sequence analysis, which revealed only sequences resembling hypothetical proteins of the host but no sequences corresponding to known viruses.

Alphaendornaviruses

Virus contigs resembling members of *Endornaviridae* were present in high numbers in all the RNA pools. After removing redundant contigs and selecting the longest contigs representing each sequence type, three distinct representative contigs of endornaviruses were determined and named Phytophthora cactorum alphaendornavirus 1–3 (PcAEV1–3; Table 2). The predicted polyprotein-encoding region of these viruses seemed to constitute the complete coding sequence. It comprised the following conserved motifs based on NCBI Conserved motif search: RdRP_2 superfamily (cl03049), Viral_helicase1 superfamily (cl26263) and Glycosyltransferase_GTB-type superfamily (cl10013). Each of the three contigs representing a distinct endornavirus included numerous single nucleotide polymorphisms (SNPs) when mapped against the RNA-seq raw data, suggesting high intraspecific variation. The alphaendornavirus contigs obtained from Trinity *de novo* assembly were of variable lengths, but the longest contigs ranged from 12.8 kb (PcAEV2 and PcAEV3) to 13.6 kb (PcAEV1). These viruses could also be observed as the dsRNA element 1 in dsRNA analysis (Fig. 1).

The near-full genome sequence of PcAEV1 was determined from isolate PhF12/18 by Sanger sequencing (Table 2). The obtained sequence was 13 363 nt long and contained a nearly complete polyprotein-coding sequence (4410 aa). The raw reads were mapped against the obtained sequence (delimiting the mapping to reads with 100% identity) in order to obtain more sequence information from the 3′ end of the virus. Notably, none of the Trinity contigs was identical to the PcAEV1 sequence obtained by Sanger sequencing from PhF12/18 complementary DNA (cDNA). The most similar Trinity contig differed from the obtained sequence by 123 nucleotide sites (most of the variability was between positions 10 963 and 11 666). Moreover, we detected 25 double peaks in the Sanger sequence chromatograms of PcAEV1 from PhF12/18 (one of which was in the RdRp region; Supplementary Table S2). These were verifiable based on raw reads mapping, where in each case one of the bases corresponded to the most similar Trinity contig, suggesting that they represented true polymorphic sites and were not related to technical issues with the Sanger sequencing. The sequence shared 36.29% aa BLASTp identity with the hypothetical protein PEV1p1 of Phytophthora alphaendornavirus 1 (PEV1) (YP_241110.1; query cover 97%), the closest endornavirus present in the database.

The near-full genome sequence (12586 b) of PcAEV2 coding for a partial polyprotein of 1477 aa was determined from isolate PhF38 (Table 2). Again, none of the Trinity contigs was identical to the obtained sequence. The Sanger sequence differed from the most similar Trinity contig by nine nucleotide sites and it contained five double peaks/bases verified by read mapping. The polyprotein shared 29.94% BLASTp identity with Erysiphe cichoracearum alphaendornavirus (YP_009552081.1, query cover 70%).

Finally, the near-full genome sequence of PcAEV3 (12675 b) was determined from isolate PhF101. The sequence had two double peaks supported by the read mapping results and differed by 23 bases from the most similar Trinity contig. The near-complete polyprotein sequence of PcAEV3 (4180 aa) shared 29.97% BLASTp identity with Erysiphe cichoracearum alphaendornavirus (YP_009552081.1, query cover 85%).

The aa sequences of PcAEV2 and PcAEV3 were 44.8% similar in global alignment, while PcAEV1 shared only 17.4% and 17.5% identity with the aa sequences of PcAEV2 and PcAEV3 respectively. Their closest sequence identities to previously known sequences were 29%–36% based on BlastP analysis of the predicted RdRp (see above).

International Committee on Taxonomy of Viruses (ICTV) sets the following species delimitation criteria for genus *Alphaendornavirus* (Valverde *et al*., 2019): 'members of different species have an overall nucleotide sequence identity below 75 %'. Based on this, the three endornaviruses detected in *P. cactorum* each represent a new species. Schematic representations of genomic organizations of PcAEV1-3 are shown in Fig. 2. Analysis of phylogenetic relationships among the three endornaviruses detected in this study and other endornavirus sequences retrieved from GenBank was conducted separately for the RdRp and helicase domains. In both cases, they were affiliated with alphaendornaviruses. PcAEV1 clustered together with PEV1, while the closely related PcAEV2 and PcAEV3 formed a cluster with Erysiphe cichoraceaum and grapevine endophyte alphaendornaviruses (Fig. 3).

Species-specific screening primers for the three alphaendornaviruses were designed (Supplementary Table S1), and cDNAs of isolates in the corresponding

Table 2. The *Phytophthora cactorum* isolates hosting alphaendornaviruses PcAEV1, PcAEV2 and PcAEV3 including GenBank accession numbers and lengths of sequenced regions.

Virus variant	Virus strain	GenBank accession	Sequence length (nt)	No of Trinity contigs found/no of mapping reads (RNAseq pool)	Additional isolates hosting viral sequence variant
AEV1_PhF12/18 [1]	*1*	MW39898	**13 363**	76/53922 (Phyto02)	PhF92, PhF114[2], PhF115, PhF116, PhF119[1], PhF2017/02[?], PhF11/18[1], PhF13/18[1], PhF14/18, PhF25/18, PhF03/19, PhF05/19, PhF21/19[2], PhF29/19, PhF30/19[1], PhF31/19, NY577[3], PhF01/19[2], PhF25/19[1], PhF26/19[1], PhF33/19, PhF34/19[1]
AEV1_ PhF02/19	*1*	MW363657	1212		PhF16/18[1], PhF2017/01[a] PhF11/18[1], PhF01/19[2], PhF25/19[1], PhF26/19[1], PhF34/19[1]
AEV1_PhF16/19	*1*	MW363660	1212		PhF15/19
AEV1_PhF23/19	*1*	MW363664	1212		PhF44
AEV1_PhF27/19	*1*	MW363665	1212		PhF108
AEV1_PhF22/19[2]	*1*	MW363662	1212		–
AEV1_PhF06/19[2]	*1*	MW363658	1212		–
AEV1_PhF18/19[1]	*1*	MW363661	1212		–
AEV1_PhF23/18[2]	*1*	MW363663	1212		–
AEV1_PhF101[a]	*1*	MW363685	1152		–
AEV1_PhF113	*1*	MW363659	1212		–
AEV1_BBA62642	*1*	MW363656	1212		–
AEV1_PhF8	*2*	MW363686	1212		PhF33[3], PhF38[2], PhF101[a,2], PhF2017/03[a], NY422[2]
AEV1_PhF9	*2*	MW363687	1198		–
AEV1_PhF2017/03[a]	*3*	MW363688	1212		PhF2017/01[a]
AEV2_PhF38	*1*	MW349899	**12 586**	20/63613 (Phyto01)	PhF33, NY422[2]
AEV2_PhF8	*1*	MW349905	1192		–
AEV2_PhF9	*1*	MW349906	1192		–
AEV2_PhF34/19	*2*	MW349907	1192		PhF05/19[1], PhF12/18[3], PhF13/18[4], PhF14/18[2], PhF25/19[2], PhF26/19, PhF29/19[2], PhF33/19[1], PhF11/18, PhF25/18[1], PhF2017/01[1], PhF2017/02[2]
AEV2_PhF16/18[1]	*2*	MW349908	1192		PhF13/18[4], PhF14/18[2]
AEV2_PhF06/19	*2*	MW349909	1192		–
AEV2_BBA62642	*3*	MW363655	1192		–
AEV3_PhF101	–	MW349900	**12 675[2]**	2/41059 (Phyto01)	PhF111[1]
AEV3_PhF12/18	–	MW363669	1218		PhF2017/01[4], PhF11/18[1], PhF13/18, PhF14/18, PhF25/18[1], PhF16/18[2], PhF02/19[1], PhF03/19[3], PhF05/19[5], PhF24/19[2], PhF25/19[3], PhF26/19[2], PhF29/19[1], PhF34/19[2]
AEV3_PhF23/19	–	MW363676	1217		PhF119, PhF114[2], PhF115[2]
AEV3_ PhF15/19	–	MW363670	1218		PhF16/19[6]
AEV3_PhF01/19[3]	–	MW363666	1218		PhF33/19[4]
AEV3_PhF113	–	MW363679	1211		AEV3_PhF92[2]
AEV3_PhF23[1]	–	MW363674	1211		–
AEV3_PhF116[2]	–	MW363680	1208		–
AEV3_PhF126[1]	–	MW363681	1204		–
AEV3_PhF2017/02[3]	–	MW363682	1211		–
AEV3_PhF07/18	–	MW363668	1084		–
AEV3_PhF23/18	–	MW363675	1219		–
AEV3_06/19[3]	–	MW363667	1191		–
AEV3_PhF18/19[4]	–	MW363671	1218		–
AEV3_PhF21/19	–	MW363672	1218		–
AEV3_PhF22/19[2]	–	MW363673	1218		–
AEV3_PhF27/19[6]	–	MW363677	1218		–
AEV3_PhF31/19[1]	–	MW363678	1208		–
AEV3_BBA61642	–	MW363683	1218		–
AEV3_NY577	–	MW363684	1218		–

The near-full genome sequences were obtained from virus variants indicated in bold.
Superscript numbers = the amount of double peaks in the RdRp sequence (checked with mapping against raw reads). The sequence sent to GenBank was obtained by choosing the more common base. Underlining: specific double peak indicating that isolate includes both AEV1_PhF12/18 and AEV1_PhF02/19 (which differ by one base) or AEV2_PhF34/19 and AEV2_PhF16/18 (which differ by two bases).
[a]In these isolates, two distinct PcAEV1 strains were detected.

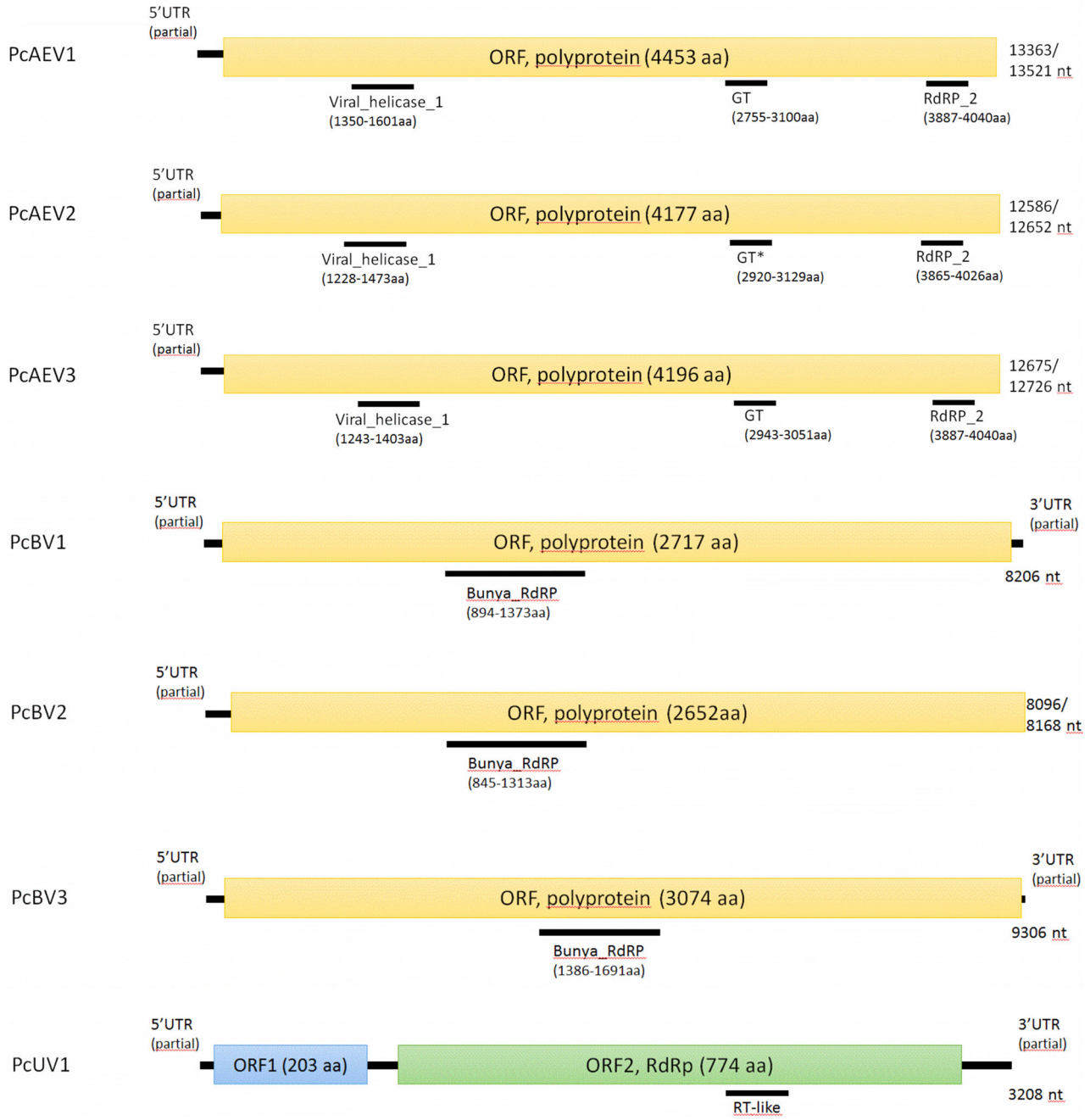

Fig 2. Schematic representations of genomes of the novel viruses detected in this study by RNA-seq. The contig-length sequences were validated by Sanger sequencing. In the cases of PcAEV1-3 and PcBV2, the obtained Sanger sequence did not cover the whole polyprotein. Mapping against raw reads allowed for the determination of the polyprotein aa sequence, but not the whole coding nt sequence due to synonymous SNPs. Nt sequence length is shown as Sanger-validated sequence/sequence obtained by mapping. The validated partial sequences are available in GenBank. The complete polyprotein lengths (end of coding sequence obtained by mapping) are given in the image, and the complete aa-sequences of PcAEV1, PcAEV2, PcAEV3 and PcBV2 are given in supplementary file S1. *GT-domain not found by NCBI conserved domain search, instead by homology comparison with PcAEV1 and PcAEV3.

RNA-seq pools, as well as the 19 additional isolates, were screened for the presence of the viruses. Altogether 50 isolates harboured an alphaendornavirus. Fourty-five (51.1%) isolates were infected with PcAEV1, 21 (23.9%) isolates with PcAEV2, and 40 (45.5%) with PcAEV3 (Table 2). For intraspecific variation in the three viruses, a region of 1212 nt from the polyprotein sequence coding for RdRp (containing RdRP_2 superfamily (cl03049) conserved domain) was amplified from cDNA of all but one (PhF07/18) isolates containing endornaviruses. Among

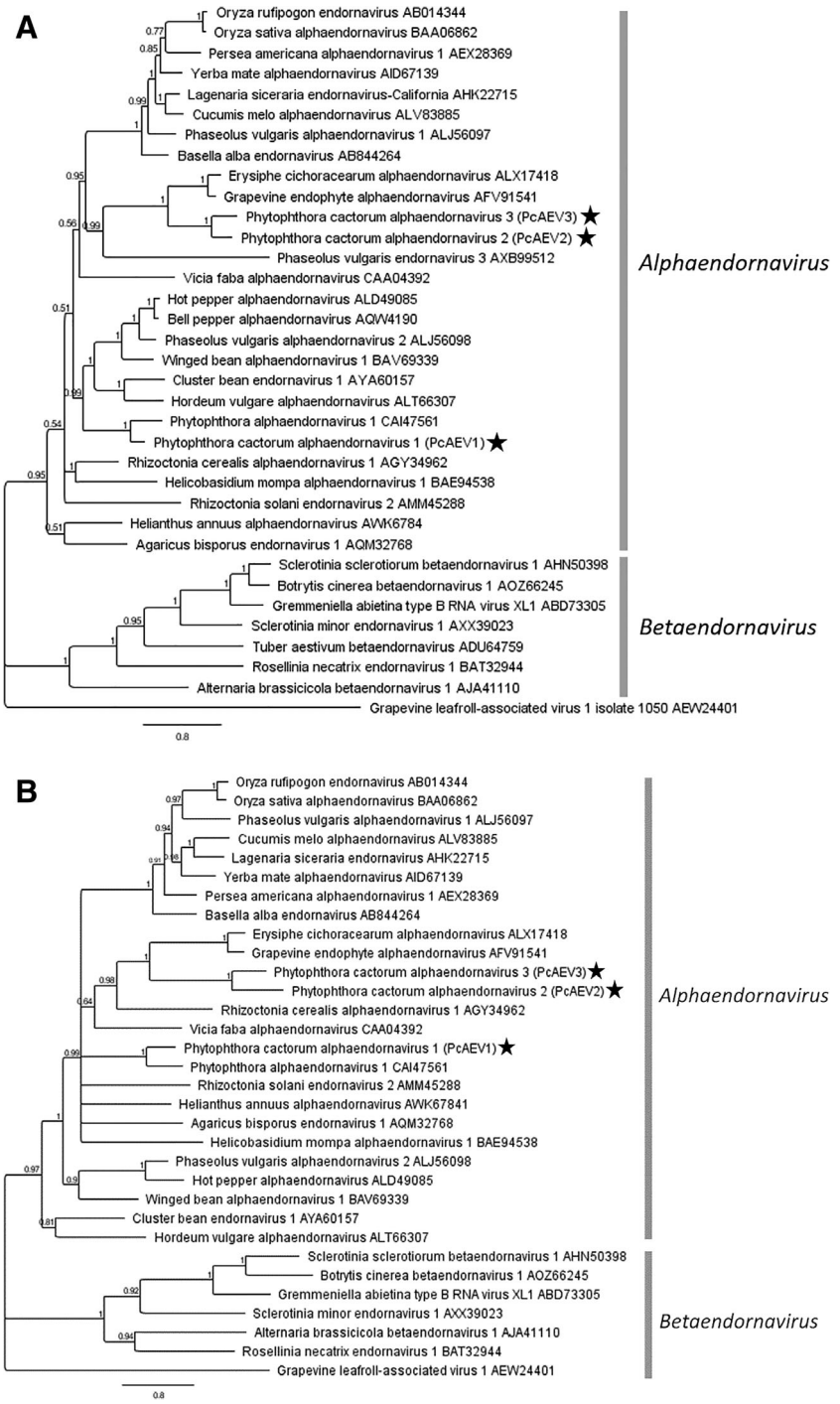

Fig 3. Phylogenetic analysis based on the RdRp (A) and helicase (B) amino acid sequences of the endornaviruses detected in this study, and those of related viruses. The sequence region included in the MAFFT alignment consisted of 444 (A) and 301 (B) aa sites. The analysis was conducted with MrBayes, using Grapevine leafroll-associated virus 1 as an outgroup. The scale bar shows 0.8 aa substitutions per site, and percentage posterior probabilities are shown at branch nodes.

the RdRp sequences of PcAEV1, three separate strains were detected, which exhibited 87.0%–88.9% nt and 97.5%–98.8% aa sequence identity between each other. Nucleotide similarities among sequence variants of strains 1 and 2 were 98.9%–100% (1–12 polymorphic sites) and 99.9% (1 polymorphic site) respectively. The sequences derived from some isolates included double peaks/bases indicating the presence of more than one sequence variant. These were checked with mapping against raw reads. The numbers of double peaks in the sequences are shown in Table 2. The alignment of PcAEV2 RdRp sequences from each isolate hosting the virus revealed three strains of the virus, which exhibited 95.5%–96.4% nt and 98.7%–99.2% aa sequence identity among each other (Table 2). Nucleotide similarities among sequence variants of strain 1 were 99.5%–99.8%

Table 3. Most of the *Phytophthora cactorum* isolates hosting the toti-like Phytophthora cactorum RNA virus 1 (PcRV1) including GenBank accession numbers and lengths of sequenced regions.

Virus variant	Virus strain	GenBank accession	Sequence length (nt)	No of Trinity contigs found/no of mapping reads (RNAseq pool)	Additional isolates hosting viral sequence variant
PcRV1_PhF04/19	1	MW349897	**5540**	13/422110 (Phyto04)	PhF09/18, PhF25/18, PhF06/19*[1], PhF07/19, PhF09/19, PhF11/19[1], PhF12/19, PhF13/19, PhF14/19, PhF24/19, PhF32/19, PhF12/18, PhF1011/18[1]
PcRV1_PhF125	1	MW472728	2327		PhF9[3], PhF90, PhF109[1], PhF130[1], PhF19/18[2], PhF19/19[1], PhF32/19, PhF21/18[1], PhF128[1]
PcRV1_PhF44	1	MW472729	2327		PhF23[1], PhF114[1]
PcRV1_PhF35/19[1]	1	MW472730	2327		PhF1010/18[1], PhF1013/18[1]
PcRV1_PhF103	1	MW472731	2327		PhF89[1], PhF118
PcRV1_PhF21/19	1	MW472732	2301		PhF119
PcRV1_PhF18	1	MW472733	2327		–
PcRV1_PhF108[2]	1	MW472734	2327		–
PcRV1_PhF110	1	MW472735	2327		–
PcRV1_PhF112[1]	1	MW472736	2327		–
PcRV1_PhF115	1	MW472737	2327		–
PcRV1_PhF126[2]	1	MW472738	2185		–
PcRV1_PhF129[1]	1	MW472739	2327		–
PcRV1_PhF16/18	1	MW472740	2327		–
PcRV1_PhF23/18	1	MW472741	2327		–
PcRV1_PhF11/18	1	MW472742	2247		–
PcRV1_PhF10/19[1]	1	MW472743	2022		–
PcRV1_PhF17/19[1]	1	MW472744	2327		–
PcRV1_PhF28/19	1	MW472745	2323		–
PcRV1_ PhF30/19	1	MW472746	2323		–
PcRV1_NY577	1	MW472752	2326		–
PcRV1_PhF38	2	MW472747	2320		PhF5, PhF33, NY422
PcRV1_PhF8[1]	2	MW472748	2320		–
PcRV1_PhF101	3	MW472749	1957		–
PcRV1_PhF22/19[1]	3	MW472750	2320		–
PcRV1_PhF2017/03	4	MW472751	2320		–
PcRV1_PhF79	5	MW472753	5654		–

The near-full genome sequences were obtained from virus variants indicated in bold.
PcRV1 sequences from 23 isolates hosting two or more virus strains were not determined (see text, Table S3). Superscript numbers = the amount of double peaks in the RdRp sequence (checked with mapping against raw reads). The sequence sent to GenBank was obtained by choosing the more common base. Underlining: sequence includes hard-to-decipher forward-direction sequence stretches at distinct positions in the RdRp sequence (around positions 137–288 and 2022–2280). In these areas the sequence was determined only by reverse-direction amplification.

(1–5 bases) and of strain 2 99.4%–99.8% (2–5 bases). Intraspecific variation among PcAEV3 RdRp sequences was relatively low, and the nucleic acid similarities among the sequence variants were 98.9%–99.9%.

Toti-like viruses

After removing redundant contigs, one distinct consensus sequence containing notable intraspecies variation was found to resemble the toti-like virus PcRV1 (Poimala and Vainio, 2020). The near-full genome sequence (5540 b) was amplified from isolate PhF04/19 (Table 3). The obtained sequence from isolate PhF04/19 had the highest identity with a 5701 nt long Trinity contig by difference in 20 nucleotide sites. All of the differing nucleotides were present in other contig sequences (19) or in raw reads only (1). The PcRV1 genome coded for a CP-RdRp fusion protein of 1580 aa, which had 90.5% aa sequence identity with PcRV1 (MN956531.1, query cover. 93%). PcRV1 is related to viruses from *Pythium* and *Plasmopara viticola*, and they form an oomycete-specific cluster of unclassified GVL-like viruses of the family *Totiviridae* in phylogenetic analysis (Poimala and Vainio, 2020, Fig. 4). The dsRNA element 2 (Fig. 1) corresponding to PcRV1 was also visible in the isolates that harboured the virus based on RT-PCR.

The partial RdRp-coding region (2326 b) was amplified from isolates hosting PcRV1. Alignments of the RdRp sequences resulted in five separate strains of the virus (Tables 3 and 5). The RdRp sequence variant PcRV1_PhF04/19 of strain 1 and PcRV1_PhF22/19 of strain 3 differed from each other by 91 nucleotides (95.4% identity; Table 5). Strain 5 differed significantly from the other four (Table 5), and it was found in only one isolate (PhF79). Strain 5 shared 99.7% nucleotide identity with the recently described PcRV1 (Poimala and Vainio, 2020). Using this previously described PcRV1 sequence as reference, we were able to obtain the

Table 4. The *Phytophthora cactorum* isolates hosting bunya-like viruses PcBV1, PcBV2 and PcBV3 including GenBank accession numbers and lengths of sequenced regions.

Virus variant	GenBank accession	Sequence length (nt)	No of Trinity contigs found/ no of mapping reads (RNAseq pool)
PcBV1_PhF79[1]	MW349901	**8206**	7/137328 (Phyto01)
PcBV1_PhF66	MW455168	2192	
PcBV1_PhF2017/03	MW455169	2192	
PcBV2_PhF79[4]	MW349902	**8096**	9/191896 (Phyto01)
PcBV2_PhF66	MW388712	2186	
PcBV2_NY422	MW388713	2184	
PcBV3_PhF79[2]	MW349903	**9306**	2/38873 (Phyto01)

The near-full genome sequences were obtained from virus variants indicated in bold.
Superscript numbers = the amount of double peaks in the RdRp sequence (checked with mapping against raw reads). The sequence sent to GenBank was obtained by choosing the more common base.

near-full genome sequence of PcRV1 strain 5 from PhF79 by mapping against raw reads. The whole polyprotein sequences from PhF79 and PhF04/19 shared 90.0% aa identity in global alignment.

Bunya-like viruses

After removing redundant contigs, three distinct consensus sequences were found to resemble bunyaviruses. These were named Phytophthora cactorum bunya-like viruses 1-3 (PcBV1-3). All the obtained contigs corresponded to the large segment coding for the RdRp. The representative Trinity contigs of PcBV1, PcBV2 and PcBV3 had lengths of 8.4, 8.3 and 9.3 kb respectively. The bunyaviruses were not visible in the dsRNA analysis. The single ORF found in these segments coded for a large 2578–3079 aa protein, which shared a 18.6%–47.8% similarity with each other in global alignment (Fig. 2; Tables 6 and 7). Isolate PhF79 was selected for the contig sequence validation by Sanger sequencing of the bunyaviruses. In the case of PcBV1, there were 103 differing nucleotide sites between the PcBV1 Sanger sequence from PhF79 and the most similar Trinity contig. The PcBV3 sequence amplified from PhF79 differed from the most similar Trinity contig by 125 bases and included four polymorphic nucleotide sites based on double peaks observed in the Sanger sequences. The near-full genome sequence of PcBV4 was obtained from a single Trinity contig. The PcBV4 sequence, which was present only in isolate PhF79, showed almost no variation in mapping against raw reads, except for two polymorphic sites in Sanger sequences, which were also found with read mapping. The aa sequences of the predicted RdRp of the three bunya-like viruses were subjected to BLASTp search, and the closest relative to the viruses PcBV1 and PcBV2 was the Halophytophthora RNA virus 6 (QLF99173.1), with which they shared 65.25% and 51.50% aa identity (query covers 69% and 69%) respectively. PcBV3 shared 39.28% (query cover 73%) identity with the Halophytophthora RNA virus 1 (QLF99168.1). Phylogenetic analysis based on partial (1100 bp) RdRp amino acid sequences of PcBV1–3 and those of related viruses revealed that the bunyaviruses detected in this study group with a yet unclassified virus group provisionally named as 'deltamycobunyaviruses' (Nerva *et al*., 2019, Fig. 5). No ICTV species delimitation criteria are yet available for this virus group, however, the identity scores are relatively low, and we consider the viruses likely to represent different species.

Bunya-like viruses were found to occur in only four isolates. A 2184–2192 nt long segment including Bunyavirus RNA dependent RNA polymerase (pfam04196) conserved domain was amplified from the isolates hosting them. The distance matrixes of the bunyavirus sequences are shown in Tables 6 and 7.

In order to search for putative second genome segments of bunya-like viruses, our dataset was compared to that of Botella *et al*. (2020) describing bunyaviruses of *Halophytophthora*. Translated BLASTx was conducted between Trinity contig libraries of pool Phyto01 and the RNA-seq pool from Botella *et al*. (2020), and all contigs over 1 kb with significant matches were examined after depleting host (*Phytophthora cactorum* GCA_003287315.1) and known viral sequences. From the 37 contigs with significant hits, the majority were found to be of host (29) or bacterial (6) origin, and/or the query coverage was extremely low (2), making them poor candidates for viral segments.

'Ustivirus'

A 3208 nt viral contig sequence was detected in RNA-seq pool Phyto01, in which only two SNP were found by mapping against raw reads (Table S2). The sequence shared 32.77% BLASTx identity with the putative RdRp of Purpureocillium lilacinum nonsegmented virus 1 (query cover 43%), which is a member of an unclassified virus group closely related to partitiviruses with the proposed name 'Ustivirus' after Ustilaginoidea virens nonsegmented virus-1, one of the first characterized members of the clade. Thus, the new virus was named Phytophthora cactorum usti-like virus 1 (PcUV1, GenBank accession MW349904). Phylogenetic tree based on the PcUV1 RdRp amino acid sequence and those of related viruses are shown in Fig. 6. Only one representative contig for PcUV1 was found, to which 88 864 reads were mapped. PcUV1 was found in only

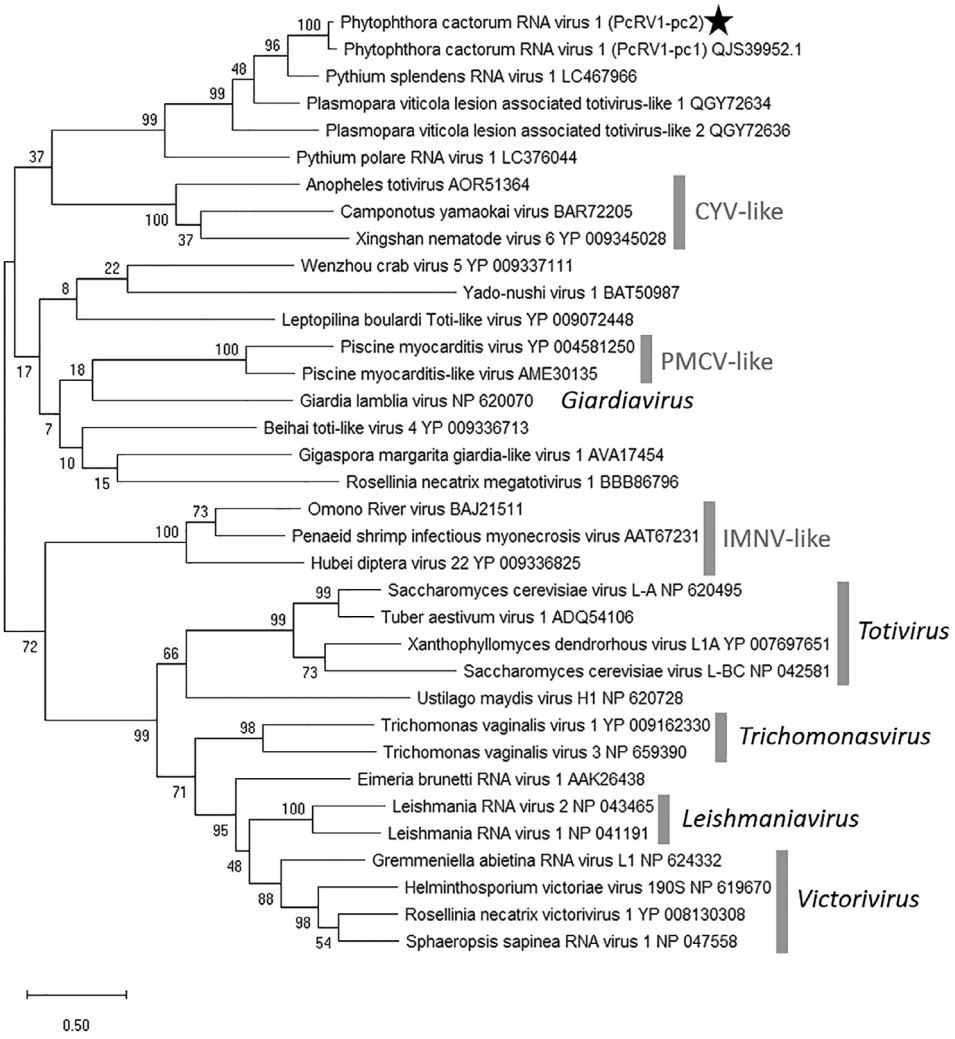

Fig 4. Phylogenetic analysis based on the RdRp amino acid sequences of the Phytophthora cactorum RNA virus 1 (PcRV1) and related viruses. The analysis was conducted by using the Maximum Likelihood method, with LG substitution model, 1000 bootstrap replicates and 95% partial deletion.

Table 5. Nucleotide (above diagonal) and amino acid (below diagonal) identities between RdRp sequences of PcRV1 strains 1–5.

	PcRV1_PhF04/19 *variant 1* (%)	PcRV1_PhF2017/03 *variant 4* (%)	PcRV1_PhF22/19 *variant 3* (%)	PcRV1_PhF38 *variant 2* (%)	PcRV1_PhF79 *variant 5* (%)
PcRV1_PhF04/19 *variant 1*	–	98.7	98.5	98.7	90.4
PcRV1_PhF2017/03 *variant 4*	95.4	–	98.7	99.0	90.0
PcRV1_PhF22/19 *variant 3*	96.4	95.4	–	99.5	89.7
PcRV1_PhF38 *variant 2*	96.3	95.5	96.4	–	89.8
PcRV1_PhF79 *variant 5*	81.4	81.7	81.1	81.6	–

The sequence lengths were 2320 nt and 773 aa.

one isolate (PhF79) by RT-PCR screening. PhF79 was also the only isolate that hosted the dsRNA element 3 (Fig. 1). The sequence of PcUV1 included two ORFs separated by 63 nt, coding for predicted proteins of 203 aa and 774 aa, the latter of which coded for the putative RdRp (Fig. 2).

Possibility of genomic integration of viral sequences

The screening primers for the endorna-, bunya-, totilike- and ustiviruses detected in this study (Table S1) were used for PCR amplification using DNA templates of three host isolates (or one in the case of PcUV1) harbouring each virus. The viral sequences did not amplify from the DNA templates, indicating that the detected contigs originated from RNA viruses instead of genome-integrated DNA.

Virus incidence and coinfections among viruses of different genera, species and viral strains

The *P. cactorum* collection analysed during this study was extremely rich in viruses, since only one seemingly virus-free isolate was found (PhF08/19) and up to six viruses (including different strains of the same virus) were found to infect a single isolate of *P. cactorum* (Table S3). The recently described PcRV1 (Poimala and Vainio, 2020) was the most abundant virus found in this study, since it was detected from 83 of the 88 isolates (96.5%). Furthermore, 50 out of 88 (62.5%) isolates hosted at least one alphaendornavirus. The most common was PcAEV1, which was found in 45 isolates, whereas PcAEV3 and PcAEV2 were found in 41 and 21 isolates respectively. The bunyaviruses (PcBV1–3) were more infrequent; they were hosted by only four isolates, where PcBV1 and PcBV2 were found in three and PcBV3 in one isolate. The putative 'ustivirus' PcUV1 was found only in a single isolate (PhF79).

Coinfections among viruses of different genera were common. The most prevalent virus PcRV1 often co-occurred with alphaendornaviruses. Out of the four isolates hosting bunyaviruses, two were coinfected with alphaendornaviruses and three with PcRV1. The only isolate hosting PcUV1 also hosted three bunyaviruses and PcRV1 (Table 2, Table S3).

Viruses of different species but representing the same genus were also often found in coinfections. Two isolates hosted more than one bunyaviruse (PhF79 hosted all three, while PhF66 hosted PcBV1 and PcBV2). The other two isolates hosted a single bunyaviruse together with one to two alphaendornaviruses. Furthermore, out of the 50 isolates hosting endornaviruses, an infection of a single virus was found only in eight isolates; three hosted only PcAEV1, while five hosted only PcAEV3. Sixteen isolates hosted all three alphaendornaviruses, while five hosted both PcAEV1 and PcAEV2, and 21 both PcAEV1 and PcAEV3. Interestingly, PcAEV2 always coinfected the host with PcAEV1. Moreover, PcAEV2 strain 2 was always paired with PcAEV1 strain 1 (14 isolates) or strain 3 (1 isolate, PhF2017/01). Instead, PcAEV2 strain 1 was always paired with PcAEV1 strain 2 (five isolates) (Table 2, Table S3). Furthermore, the rare PcRV1 strain 2 was found only in coinfection with PcAEV1 strain 2 and PcAEV2 strain 1 (three isolates). Bunyaviruses were not found in coinfections with the most common strains of the endornaviruses (Table 2, Table S3). Interestingly, isolate PhF79, which hosted three bunyaviruses was also the only isolate hosting the putative ustivirus PcUV1 as well as the unique strain 5 of the toti-like virus PcRV1. Based on a small-scale Random Amplified MicroSatellite (RAMS) -analysis of selected *P. cactorum* isolates (as done in Hantula *et al*., 2000), isolates PhF66 and PhF79 carried the marker CGA330 specific to birch-type

Table 6. Amino acid identities between the whole polyprotein sequences of the three bunya-like viruses (2578–3079 aa).

	PcBV1 (PhF79) (%)	PcBV2 (PhF79) (%)	PcBV3 (PhF79) (%)
PcBV1 (PhF79)	–	47.8	18.6
PcBV2 (PhF79)	47.8	–	19.0
PcBV3 (PhF79)	18.6	19.0	–

Isolate names are given in parenthesis.

Table 7. Nucleotide (above diagonal) and amino acid (below diagonal) identities between the RdRp sequences of the three bunya-like viruses from all isolates hosting them.

	PcBV1 (PhF66) (%)	PcBV1 (PhF79) (%)	PcBV1 (PhF2017/03) (%)	PcBV2 (PhF66) (%)	PcBV2 (PhF79) (%)	PcBV2 (NY422) (%)	PcBV3 (PhF79) (%)
PcBV1 (PhF66)	–	97.2	94.9	62.2	62.1	62.1	41.0
PcBV1 (PhF79)	99.5	–	94.9	61.8	62.2	62.0	40.5
PcBV1 (PhF2017/03)	99.2	99.2	–	62.4	62.7	62.5	40.0
PcBV2 (PhF66)	65.7	65.6	65.7	–	97.0.1	96.4	39.4
PcBV2 (PhF79)	65.5	65.4	65.5	99.0	–	98.2	39.5
PcBV2 (NY422)	65.7	65.5	65.7	99.0	99.5	–	39.7
PcBV3 (PhF79)	26.3	26.1	26.0	25.8	25.9	25.8	–

Isolate names are given in parenthesis.

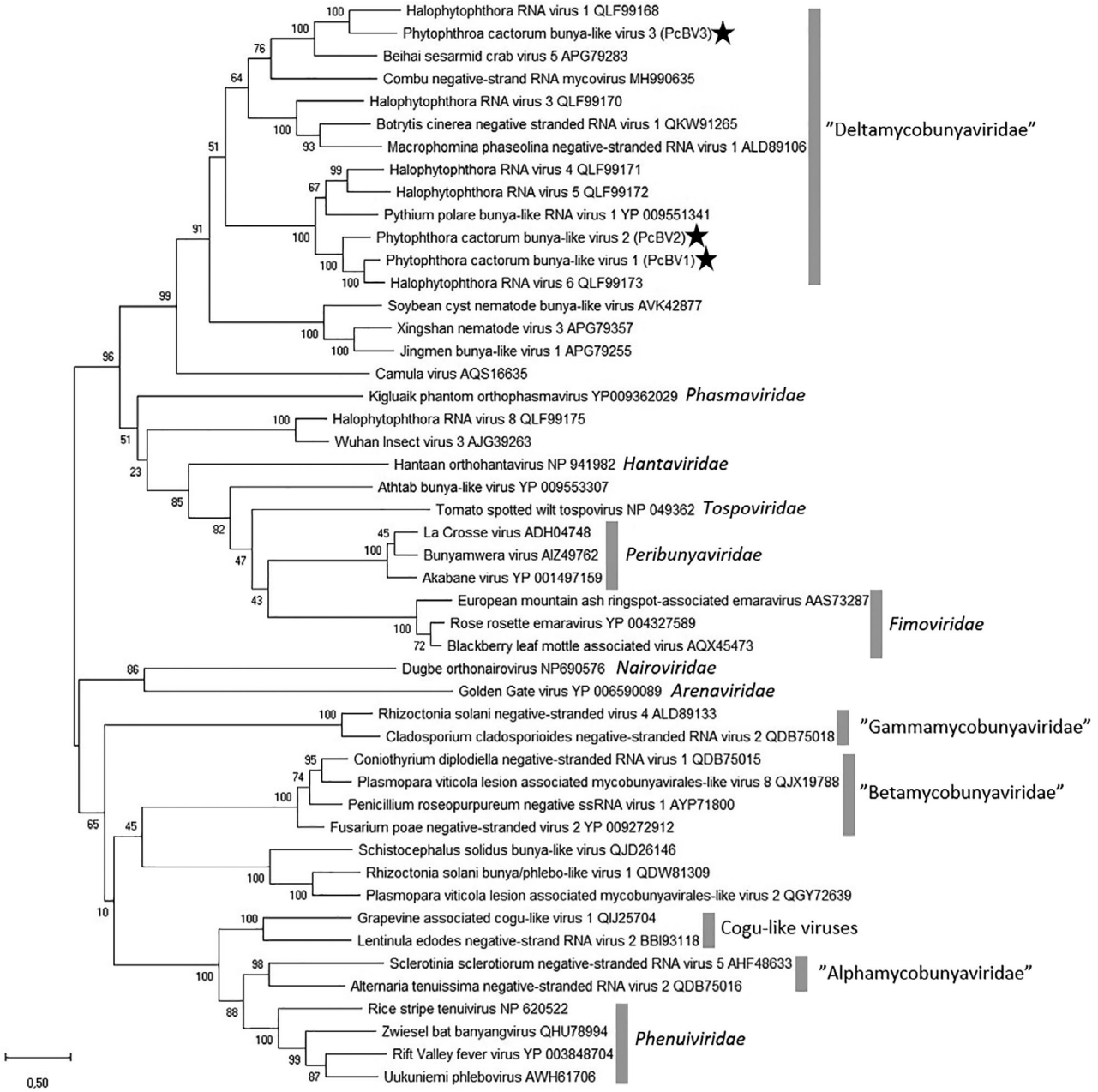

Fig 5. Phylogenetic analysis based on the RdRp amino acid sequences (752 aa) of the Phytophthora cactorum bunyaviruses (PcBV1-3) found in this study and those of related viruses. The analysis was conducted by using the Maximum Likelihood method with LG substitution model, 1000 bootstrap replicates and 95% partial deletion.

P. *cactorum* isolates (data not shown) although they were isolated from a strawberry plant.

Three isolates (namely, PhF101, PhF2017/01 and PhF2017/03) seemed to harbour sequences of two PcAEV1 strains. A 942 b RdRp segment of PcAEV1 strains 1 and 2 detected from isolate PhF101 shared 87.8% nt and 97.2% aa identity. Moreover, the PcAEV1 strains 1 and 3 (1314 b) detected from isolate PhF2017/01 shared 86.6% nt and 96.8% aa identity, and a 841 b RdRp segment of strains 2 and 3 from isolate PhF2017/03 shared 88.8% nt and 97.9% aa identity. Furthermore, the PcRV1 RdRp Sanger sequences of 21 isolates contained multiple double peaks. The positions of the double bases indicated that these isolates contained at least two sequences; one of strain 1 (or similar), and one of strain 3 (or similar). All double peaks differentiating strain 1 and strain 3 were not present in all sequences. These 21 isolates were

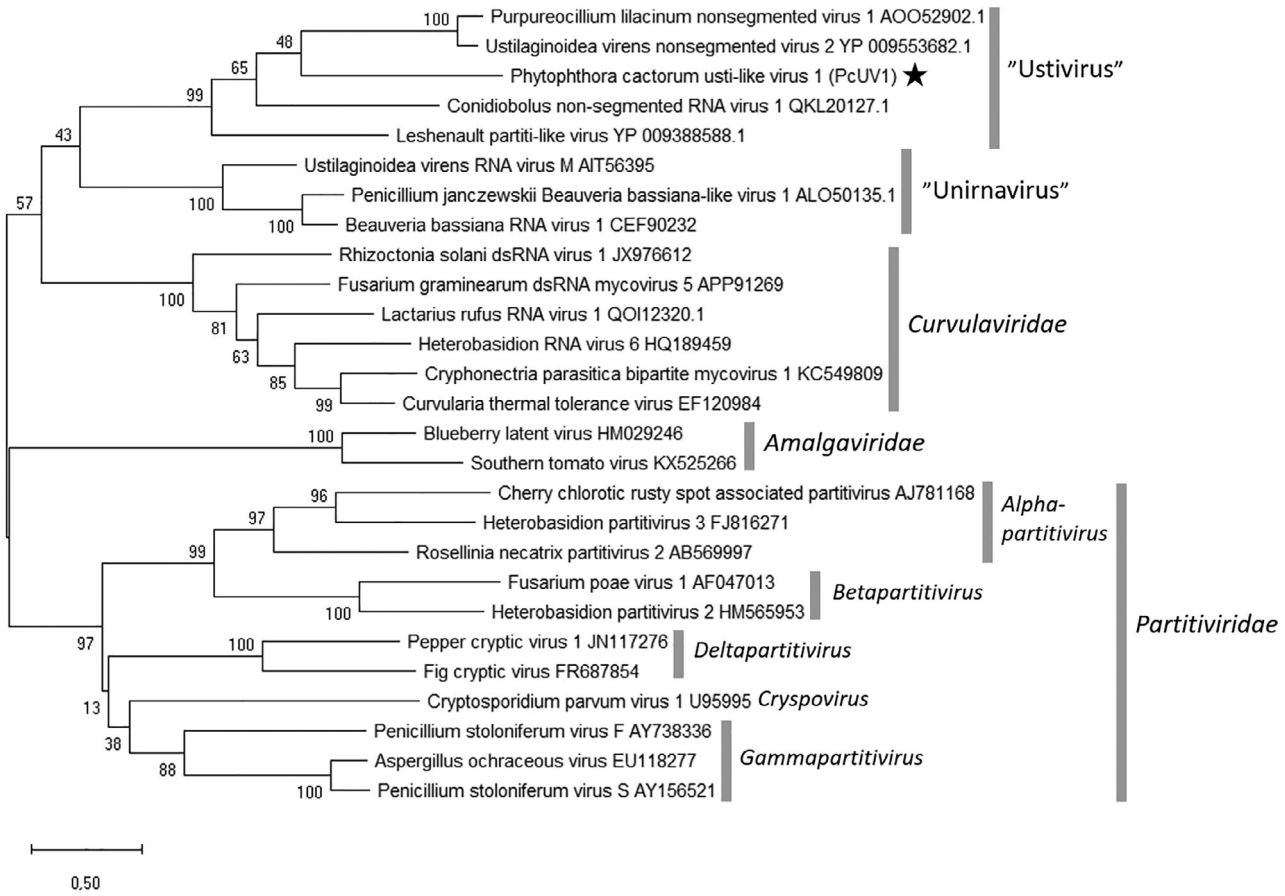

Fig 6. Phylogenetic analysis based on the RdRp amino acid sequences of Phytophthora cactorum usti-like virus 1 (PcUV1) found in this study and those of related viruses. The analysis was conducted by using the Maximum Likelihood method, with LG substitution model, 1000 bootstrap replicates and 95% partial deletion.

PhF01/19, PhF02/19, PhF03/19, PhF05/19, PhF15/19, PhF16/19, PhF18/19, PhF23/19, PhF25/19, PhF26/19, PhF27/19, PhF29/19, PhF31/19, PhF32/19, PhF33/19, PhF34/19, PhF12/18, PhF13/18, PhF14/18, PhF2017/01, PhF2017/02, PhF107 and PhF111 (Table S3).

Temporal and geographical occurrence of virus strains and variants

Most of the virus species and their strains were found in more than one isolate. The only exceptions were PcAEV2 strain 3 and PcRV1 strains 4 and 5, which were found only in a single isolate. The first was detected in the German isolate from 1971 and the latter two in Finnish isolates from years 2017 and 1991 respectively. We investigated whether there were any virus species or strains that seemed to persist through the sampling years and found that PcAEV3 was present in the Finnish *P. cactorum* isolates from 1992 to 2019. However, it was also found in the USA in the 1990s and in Germany in 1971. The most common strain of PcAEV1, strain 1, was also already present in the German isolate from 1971. It was detected in the Finnish population in 1993 as a coinfection with AEV1 strain 2, which was found only in old isolates prior to 1994, in addition to one US isolate from an unknown time in the 1990s. However, none of the sequence variants from Finnish isolates was identical to the German one. Instead, identical sequence variants of PcAEV1, PcAEV2 and PcRV1 were found among isolates from the USA and Finland (Table 2). The Finnish isolates Ph33 and PhF38 isolated in 1993 in Finland and NY422 isolated in the 1990s in the USA harboured identical sequence variants of PcAEV1 (PcAEV1_PhF8), PcAEV2 (PcAEV2_PhF38) and PcRV1 (PcRV1_PhF38). The viral composition of NY422 thus differed from the two Finnish isolates only by the additional unique bunyavirus (PcBV2). PcAEV1 strain 1 persisted in the Finnish strawberries until 2019. PcAEV2 strain 1 was also found only in isolates from 1993 and before, whereas strain 2 was observed only among isolates collected since 2017, and strain 3 was only found in the

German isolate from 1971. In the case of the toti-like virus PcRV1, strains 1, 2, 3 and 5 were already present in isolates from 1990 to 1993. Since then, the isolates from 1997 to 2019 contained almost exclusively either strain 1 or a combination of strains 1 and 3 (possibly containing also intermediates). Strain 3 was detected alone only once in 2019, and the only isolate hosting strain 4 was from 2017 (Fig. 7, Table S3).

Most of the viral species, as well as virus strains, were also detected from more than one location, and none of the viruses present in more than one isolate was detected only in a single location. However, PcAEV2 strain 2 which appeared in 2017, was detected only in Kuopio and Suonenjoki, located about 50 km apart. PcAEV1 strain 1, PcAEV3 and PcRV1 strain 1 were detected as far as around 570 km apart in Pohja and in Northern Ostrobothnia. The strawberry sampling locations and isolation years (when applicable) of the *P. cactorum* isolates are shown in Fig. 7.

Discussion

This study describes the virus population hosted by *Phytophthora cactorum* isolates collected from diseased strawberry plants in Finland through almost three decades (1990–2019). One isolate from Germany and two from the USA were also included in order to decipher the possibility of global occurrence of viruses. The incidence of viruses in strawberry isolates of *P. cactorum* was unexpectedly high, nearly 100%. In *P. infestans*, viral dsRNAs have been found in nine out of 22 isolates (41%; Cai *et al*., 2009), and endornavirus (PEV1) incidence in *P. ramorum* and *P.* taxon Douglas Fir has been found 20% (7/35) and 100% (4/4) respectively (Kozlakidis *et al*., 2010). The virus community in the present study included three alphaendornaviruses ((+) ssRNA), three bunya-like viruses ((−)ssRNA), one usti-like virus (dsRNA) and a toti-like virus (dsRNA). While alphaendornaviruses have been previously reported in *P. ramorum* and *P.* taxon Douglas fir (Kozlakidis 2010), this is the first study describing bunya- and usti-like viruses in the genus *Phytophthora*, and the first report of an usti-like virus in oomycetes. In genus *Halophytophthora*, however, bunyaviruses seem to be very common and diverse (Botella *et al*., 2020). Members of the virus group provisionally named 'Ustiviruses' are related to members of family *Partitiviridae* but have only one genome segment. Ustiviruses have so far been detected in ascomycetes of Sordariomycota (*Ustilaginoidea virens*, *Purpureocillium lilacinum*, *Nigrospora oryzae*; Zhang *et al*., 2018, Herrero, 2016, Zhou *et al*., 2016), the basal fungus

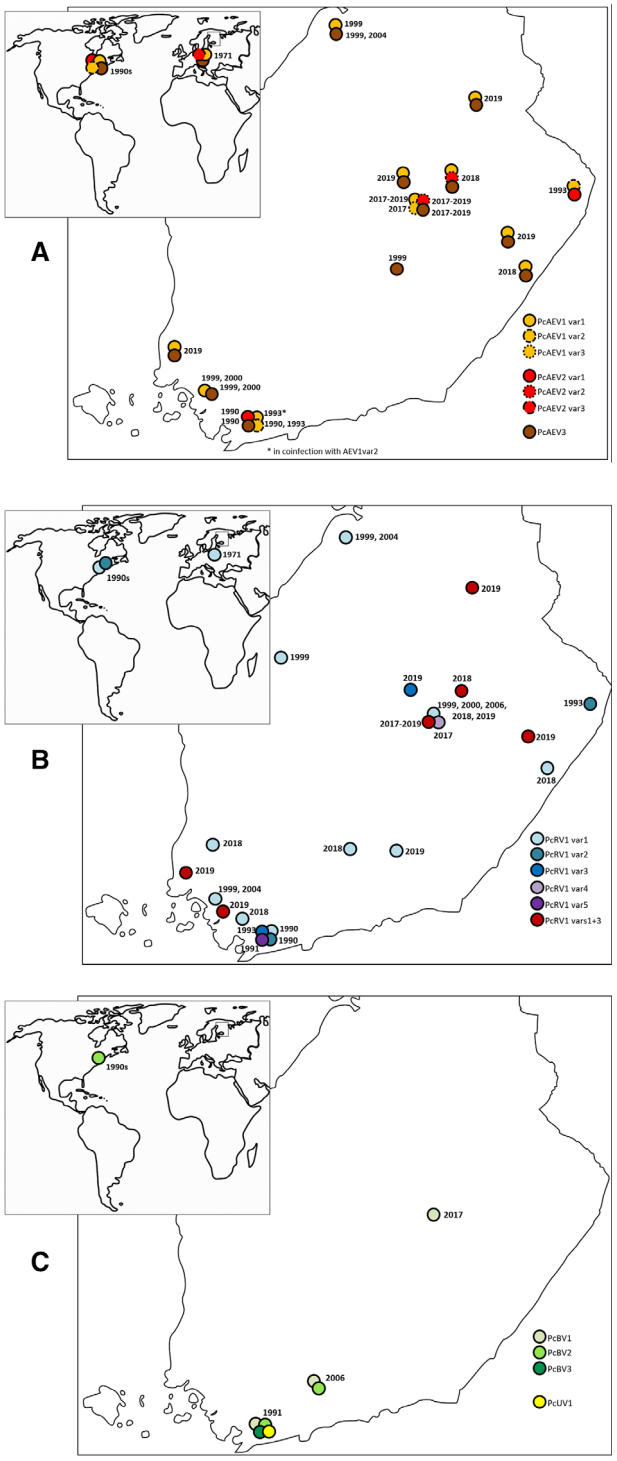

Fig 7. Isolation years and locations of *Phytophthora cactorum* isolates of this study, and the virus species and strains detected in them.
A. Phytophthora cactorum alphaendornaviruses 1–3 (PcAEV1-3), B. Phytophthora cactorum RNA virus 1 (PcRV1), C. Phytophthora cactorum bunyaviruses 1–3 (PcBV1-3) and Phytophthora cactorum usti-like virus 1 (PcUV1).

Conidiobolus sp. (Zoopagomycota; Yang *et al*., 2020) and a metatranscriptomic mosquito pool (Shi *et al*., 2017). The toti-like virus PcRV1 recently described from *P. cactorum* originating from birch (Poimala and Vainio, 2020) was found extremely frequent among the isolates from strawberry.

In the present study, viral coinfections were found to be very common in the *P. cactorum* isolates. Mixed infections were found between endorna- and toti-like viruses, and both genera were also found in coinfections with bunyaviruses. The usti-like virus was found in coinfection with bunyaviruses and PcRV1. Only four isolates of this study hosted bunya-type viruses. They did not coinfect the host with the most common strains of PcRV1, PcAEV1 or PcAEV2, instead they were found in mixed infections with rare and unique strains 2, 4 and 5 of PcRV1 as well as the uncommon strains 2 and 3 of PcAEV1 and strain 2 of PcAEV2. These coinfection patterns may be a result of virus–virus competition, which puts a selective pressure on the viruses present, and thus accelerates diversification. On the other hand, mycoviruses may also alter each other's transmission frequencies. For example, Sclerotinia sclerotiorum mycoreovirus 4 has been found to facilitate the transmission of other mycoviruses of, e.g. genera *Sclerodarnavirus* and *Mitovirus* across vegetatively incompatible groups (Wu *et al*., 2017). An extreme example of virus interplay is the yado-kari virus, a capsidless (+)ssRNA virus, that is trans-encapsidated by a coinfecting totivirus, Yado-nushi virus and replicating like a dsRNA virus (Zhang *et al*., 2016).

Viral coinfections of viruses representing different families (a narnavirus and an unclassified virus related to the proposed genus 'Fusagraviridae') have also been found in *P. infestans* (Cai *et al*., 2012, 2013). However, in the present study, viruses of the same genera are also commonly found in mixed infections. This has been observed in, e.g. mitoviruses of the Dutch Elm Disease fungus *Ophiostoma novo-ulmi* (Hong *et al*., 1999), partitiviruses of *Ceratobasidium* spp. (Ong *et al*., 2017) and endornaviruses of mycorrhizal symbionts of orchids (Ong *et al*., 2016). Coinfecting Heterobasidion partitiviruses can also affect each other's transmission frequency or suppress virus-induced negative effects on host growth (Kashif *et al*., 2019; Hantula *et al*., 2020). In the present study, two or three different alphaendornavirus species often coexisted in the same hosts. Interestingly, PcAEV2 was always detected in a mixed infection with PcAEV1, suggesting that the incidence of PcAEV2 is affected by PcAEV1.

Distinct strains of a single virus species are commonly mutually exclusive in a host. For example, *Oryza rufipogon* virus and *Oryza sativa* virus, two endornavirus species of *Oryza* (rice) species that shared 80% aa sequence identity, could not be made to coinfect a single host (Moriyama *et al*., 1999). Mutual exclusion of conspecific viral strains is also a common criterion for species delimitation (King *et al*., 2011). In contrast to these results, we observed three *P. cactorum* isolates that harboured two strains of PcAEV1. These shared ca. 97%–98% aa RdRp sequence identity with each other, which is notably higher than the species delimitation criteria for endornaviruses. To our knowledge, coinfection of a single host with two very similar but distinct strains of a mycoviral or oomycete species has not been reported earlier. Heterogenous Bell pepper endornavirus sequences have been detected as SNPs in Trinity and Velvet contigs, but the considerably lower variation compared to the strains of this study can be interpreted as quasispecies variation (Jo *et al*. 2017). In the present study, we also detected 21 cases of two or more highly similar strains of PcRV1 infecting a single host (detected by specific double peaks in the sequence). It should be noted that *Phytophthora* hyphae are aseptate, therefore potentially allowing efficient distribution and stability of viruses within the mycelium.

In the case of alphaendornaviruses, bunyaviruses and PcRV1, the common occurrence and variable nature of the viral sequences led to the formation of artificial contig sequences none of which were identical to the validated Sanger sequences of the viruses. The differences seemed to result from the presence of polymorphic nucleotide sites as well as *in silico* misassembly due to the presence of identical sequence stretches, resulting in the formation of chimaeric contigs. This finding highlights the uncertainty involved in the *de novo* contig assembly of abundant and variable virus sequences from RNA-seq data and emphasizes the importance of viral sequence validation by Sanger sequencing for further biological or taxonomical considerations of a virus species. As virus classification is currently acceptable also based on metagenomic sequences (provided that the biological characteristics can be inferred from the sequence data; Simmonds *et al*., 2017), these issues should be taken into consideration.

Currently, the majority (about 90% in 2016) of strawberry planted in Finland originate from Central Europe, mostly from the Netherlands, and crown rot is transported in the planting material from the country of origin to the planting locations (Kumpula *et al*., 2020). The disease becomes evident quickly during the first growing season, and sometimes disease incidence and mortality are very high in the imported runner plant lots. *Phytophthora cactorum* can also persist in the soil and infect new seedlings in the following few years (Eikemo *et al*., 2003a, 2003b). Infections originating from the soil are thought to be more sporadic and usually not significant in scale in Finland, however, the incidence of *P. cactorum* in Finnish

strawberry farm soils has not been studied. The planting material is usually renewed once in 3–4 years (Kumpula *et al*., 2020). Therefore, it can be assumed that also the majority of viruses are not from the putative local *P. cactorum* population residing in the soils. The high virus incidence in the strawberry populations of *P. cactorum* may indicate that the pathogen population is well established in the production facilities in Europe, and opportunities for virus transfer between the residing *P. cactorum* strains have been many. Virus curing from *Phytophthora in vitro* has been challenging (Cai and Hillman, 2013), therefore it is also possible that the viruses in the aseptate *Phytophthora* hyphae are not lost easily in the production facilities and strawberry fields.

Prior to 1995, when Finland joined the EU, the import of strawberry growing material to Finland was very infrequent (Kumpula *et al*., 2020). Thus, the isolates collected 1990–1993 may represent the population present in the Finnish runner plant production, and the changes observed in virus population between 1993 and 1997 (replacement of the endornaviruses with new strains and change in the prevalence of toti-like virus strains) might reflect the change in the origin of the runner plants. The three isolates from year the 2017 hosted rare strains of PcAEV1 and PcRV1 (PcAEV1 strain 3, PcRV1 strain 4 and PcBV strain 3), as well as one bunya-like virus (PcBV1) very rarely found in the material. This might also be due to a change in the runner plant origin location, since these rare strains were not found in earlier or later isolates (Fig. 7; Tables 1–4, Table S3).

The most common strains of PcAEV1, PcAEV3 and PcRV1 were present also in the German and the US isolates. These strains have thus been present in the *P. cactorum* population infecting strawberry since at least 1971. The US isolates likely come from the Eastern USA (Prof. Wilcox lab; Hantula *et al*., 2000) where crown rot of strawberry was first detected in 1988 (Wilcox, 1989). The two US isolates did not host the same strains of any virus species found in them. However, all virus species, their strains and in some cases also the sequence variants found in the USA were also present in the Finnish isolates from the 1990s, indicating that the virus population was similar in the United States and European host populations at the time, and suggesting for a transmission of *P. cactorum* between the two continents. Studies have shown a high genetic homogeneity in European *P. cactorum* isolates from strawberry (Cooke *et al*., 1996; Lilja *et al*., 1998) but considerably higher variation in the North American population (Hantula *et al*., 2000). Since the US isolates NY577 and NY422 and European isolates from strawberry have also been shown to be quite closely related by genetic fingerprinting (Hantula *et al*., 2000), NY577 and NY422 could represent the crown rot population that was originally imported from North America to Europe. It would be of interest to screen more recent US isolates to obtain information on which viruses are currently present. Hantula *et al*., 2000 also showed that *P. cactorum* from leather rot and crown rot of strawberry are not genetically differentiated from each other. In this study, the four leather rot isolates did not host differing viruses from the crown rot isolates, providing evidence that the two diseases are not caused by distinct oomycete-virus combinations. In addition, isolates originating from different strawberry cultivars did not show differing virus pools (Table S3).

Isolate PhF79 from the year 1991 in Pohja was especially interesting, since it hosted a mixed infection by three bunyaviruses, the only usti-like virus and a strain of PcRV1 which was nearly identical to the previously described PcRV1 from *P. cactorum* infecting birch but clearly distinct from the remaining PcRV1 strains. PhF66 also differed from other isolates by hosting only bunyaviruses. Notably, *P. cactorum* isolates from different plant species in Finland are genetically differentiated (Hantula *et al*., 2000). The abundance of bunyaviruses, presence of ustiviruses as well as lack of endornaviruses have been detected in a few *P. cactorum* isolates from woody hosts (Poimala *et al*. unpublished). The 'birch-isolate-like' virus pools of PhF79 and PhF66 may indicate that they are genetically more similar to the birch population of *P. cactorum*. Our small-scale RAMS-profiling with one primer also suggested the same. Previously, isolates from Silver birch have been unable to cause crown rot symptoms in strawberry, but strawberry isolates have caused necrotic lesions on *B. pendula* (Hantula *et al*., 1997; Eikemo *et al*., 2004). The isolates PhF79 and PhF66 have been isolated from strawberry crown rot symptoms, however, the host history of these isolates remains unknown. Future studies are needed to decipher whether host populations from birch and strawberry can be differentiated based on their virus communities, which would give further support for the limited genetic exchange between the populations.

Experimental procedures

Microbes and their maintenance

Fourty-nine isolates were collected from diseased strawberry plants in several Finnish farms in 2018 and 2019 and identified in the Natural Resources Institute Finland (Luke). Additional isolates were obtained from Luke's culture collections, including one German and two US isolates. All isolates are listed in Table 1 along with the year and country of isolation and strawberry cultivar, if known. The isolates whose origins are known are from 42 separate strawberry farms across Southern and Central Finland, and their collection sites are listed in Table 1. The

isolates were maintained on potato dextrose or malt extract agar plates prior to molecular analyses to detect possible viral infections.

Screening for dsRNA by cellulose affinity chromatography

To determine the presence of dsRNA and to visualize banding patterns in the *P. cactorum* isolates, total dsRNA was extracted from 45 isolates using CF11 cellulose affinity chromatography (Table 1). The isolates were grown on modified orange serum agar on cellophane membranes, and dsRNA was extracted from circa 2.5 g mycelia as described in Vainio *et al*., 2011 and visualized in agarose gel electrophoresis.

Total RNA extraction and RNA-seq

Prior to total RNA extraction, the isolates were grown on modified orange serum agar on cellophane membranes. The mycelia were harvested after 2 weeks, freeze-dried and ground in liquid nitrogen. The mycelia of the isolates of RNA-seq pool Phyto04 were ground in lysis buffer in sand tubes using FastPrep homogenizer. Total RNA was extracted using Spectrum plant total RNA kit (Sigma-Aldrich, St Louis, MO, USA). RNAs were quantified with a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA). Ten (pools Phyto01, 02) or 22 (pools Phyto03, 04, 05) RNA samples were pooled for each of the five RNA-seq pools, which contained 1 μg of RNA from each isolate. The pools were sent to a sequencing facility (Macrogen, Seoul, Rep. of Korea), where ribosomal RNA was depleted (Ribo-ZeroTM Gold Kit, Epicentre, Madison, USA), cDNA libraries were built (TrueSeq total RNA sample kit, Illumina) and sequencing was performed using an Illumina NovaSeq6000 system generating stranded paired-end sequences.

Bioinformatics

The *de novo* contig assembly of Illumina-generated raw reads was made with Trinity (version 2.8.5.) (Haas *et al*., 2013) as detailed in Nerva *et al*. (2018). Before the assembly, the reads were cleaned using Trimmomatic including quality-based and adapter filtering. The assembled contigs were then compared against a custom viral proteins database using BlastX to search for contigs having homology with virus sequences. Contigs with high similarity to host proteins (*Phytophthora cactorum* GCA_003287315.1) were depleted *in silico* from the dataset. The analyses were conducted at the ICT Center for Science with the aid of the Puhti supercomputer. Geneious R10 (Biomatters, New Zealand) was used to map raw reads to selected contigs and visualize the results. Candidate viral contigs were further analysed manually using NCBI BlastN and BlastX to eliminate potentially remaining host-derived sequences.

Identification of virus-infected isolates

cDNA was synthesized from each individual RNA sample included in the RNA-seq pools using random hexamer priming with Revert Aid reverse transcriptase (Thermo Scientific) following the manufacturer's recommendations. The presence of putative viruses detected by RNA-seq was examined among the *Phytophthora* isolates using PCR amplification with selective primer pairs for each virus group using DreamTaq DNA Polymerase (Thermo Scientific) (see Supplementary Table S1 for primer sequences). Fourty-five of these isolates had been analysed for the presence of dsRNA elements earlier using cellulose chromatography (see Table 1).

RNA was also extracted and reverse transcribed from 19 additional isolates that were not included in the RNA-seq pools for screening with the RT-PCR method (see Table 1). In these cases, total nucleic acids were isolated using phenol-chloroform extraction as described in Vainio *et al*., 2013.

Determining the near-full genomes of viruses and phylogenetic analyses

Because of the high prevalence and amount of intraspecific variation in most of the detected viruses, the *in silico* assembled contigs needed to be verified by Sanger sequencing. Therefore, a representative isolate for each putative virus species was chosen for virus genome sequence determination. Species-specific primers were designed to cover nearly the whole contig length. When variable sites indicated by double peaks in the sequence chromatograms were observed, the consensus sequence sent to GenBank was obtained by choosing the more common base as determined by mapping against raw reads. See Supplementary Table S2 for a detailed description of the variable sites.

The sequences were affiliated to previously known virus taxa based on the similarity of their RdRp encoding genome region as revealed using NCBI BlastX. Predicted RdRp sequences were aligned with the most closely related sequences available in GenBank to estimate their relatedness and the possible occurrence of novel virus species among the *P. cactorum* isolates. This assessment was based on the species delimitation criteria of the ICTV. Representatives of the RdRp sequences validated by Sanger sequencing were used for phylogenetic inference using the Maximum Likelihood method implemented in MEGA or MrBayes program implemented in Geneious R10. Sequence alignment for phylogenetic

analysis was conducted using MAFFT v7.388 and the Blosum45 substitution matrix.

Determination of intraspecific variation

To assess the level of intraspecific variation in the putative virus species, we determined a partial sequence of the RdRp encoding genome region of each virus species, and the RdRp was PCR amplified from each virus-containing isolate using species-specific primers. All the resulting sequences were manually checked for SNPs between sequences as revealed by double peaks in Sanger sequences and/or polymorphic sites in mapped RNA-seq reads with 20% mismatch.

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References

Abudurexiti, A., Adkins, S., Alioto, D., Alkhovsky, S.V., Avšič-Županc, T., Ballinger, M.J., *et al*. (2019) Taxonomy of the order Bunyavirales: update 2019. *Arch Virol* **164**: 1949–1965.

Botella, L., Janoušek, J., Maia, C., Horta Jung, M., Raco, M., and Jung, T. (2020) Marine Oomycetes of the genus *Halophytophthora* harbor viruses related to Bunyaviruses. *Front Microbiol* **11**: 1467.

Cai, G., Fry, W.E., and Hillman, B.I. (2019) PiRV-2 stimulates sporulation in *Phytophthora infestans*. *Virus Res* **271**: 197674.

Cai, G., and Hillman, B.I. (2013) *Phytophthora* viruses. *Adv Virus Res* **86**: 327–350.

Cai, G., Krychiw, J.F., Myers, K., Fry, W.E., and Hillman, B.I. (2013) A new virus from the plant pathogenic oomycete *Phytophthora infestans* with an 8 kb dsRNA genome: the sixth member of a proposed new virus genus. *Virology* **435**: 341–349.

Cai, G., Myers, K., Fry, W.E., and Hillman, B.I. (2012) A member of the virus family *Narnaviridae* from the plant pathogenic oomycete *Phytophthora infestans*. *Arch Virol* **157**: 165–1699.

Cai, G., Myers, K., Fry, W.E., and Hillman, B.I. (2018) *Phytophthora infestans* RNA virus 2, a novel RNA virus from *Phytophthora infestans*, does not belong to any known virus group. *Arch Virol* **164**: 567–572.

Cai, G., Myers, K., Hillman, B.I., and Fry, W.E. (2009) A novel virus of the late bligt pathogen, *Phytophthora infestans*, with two RNA segments and a supergroup 1 RNA-dependent RNA polymerase. *Virology* **392**: 52–61.

Chepsergon, J., Motaung, T.E., Bellieny-Rabelo, D., and Moleleki, L.N. (2020) Organize, don't agonize: strategic success of *Phytophthora* species. *Microorganisms* **8**: 917.

Chiapello, M., Rodríguez-Romero, J., Ayllón, M.A., and Turina, M. (2020) Analysis of the virome associated to grapevine downy mildew lesions reveals new mycovirus lineages. *Virus Evol* **6**: veaa058.

Cooke, D.E.L., Kennedy, D.M., Guy, D.C., Russell, J., Unkles, S.E., and Duncan, J.M. (1996) Relatedness of group I species of *Phytophthora* as assessed by randomly amplified polymorphic DNA (RAPDs) and sequences of ribosomal DNA. *Mycol Res* **100**: 297–303.

de Lima, J.G.S., Teixeira, D.G., Freitas, T.T., Lima, J.P.M.S., and Lanza, D.C.F.R. (2019) Evolutionary origin of 2A-like sequences in *Totiviridae* genomes. *Virus Res* **259**: 1–9.

Eikemo, H., Klemsdal, S.S., Riisberg, I., Bonants, P., Stensvand, A., and Tronsmo, A.M. (2004) Genetic variation between *Phytophthora cactorum* isolates differing in their ability to cause crown rot in strawberry. *Mycol Res* **108**: 317–324.

Eikemo, H., Stensvand, A., Davik, J., and Tronsmo, A.M. (2003b) Resistance to crown rot (*Phytophthora cactorum*) in strawberry cultivars and in offspring from crosses between cultivars differing in susceptibility to the disease. *Ann Appl Biol* **142**: 83–89.

Eikemo, H., Stensvand, A., and Tronsmo, A.M. (2003a) Induced resistance as a possible means to control diseases of strawberry caused by *Phytophthora* spp. *Plant Dis* **87**: 345–350.

Erwin, D.C., and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*: St Paul, MN, USA: American Phytopathological Society (APS Press).

Grasse, W., and Spring, O. (2017) SsRNA viruses from biotrophic Oomycetes form a new phylogenetic group between *Nodaviridae* and *Tombusviridae*. *Arch Virol* **162**: 1319–1324.

Haas, B., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J., *et al*. (2013) De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat Protoc* **8**: 1494–1512.

Hacker, C.V., Brasier, C.M., and Buck, K.W. (2005) A double-stranded RNA from a *Phytophthora* species is related to the plant endornaviruses and contains a putative UDP glycosyltransferase gene. *J Gen Virol* **86**: 1561–1570.

Hantula, J., Lilja, A., Nuorteva, H., Parikka, P., and Werres, S. (2000) Pathogenicity, morphology and genetic variation of *Phytophthora cactorum* from strawberry, apple, rhododendron, and silver birch. *Mycol Res* **10**: 1062–1068.

Hantula, J., Lilja, A., and Parikka, P. (1997) Genetic variation and host specificity of *Phytophthora cactorum* isolated in Europe. *Mycol Res* **101**: 565–572.

Hantula, J., Mäkelä, S., Xu, P., Brusila, V., Nuorteva, H., Kashif, M., *et al*. (2020) Multiple virus infections on *Heterobasidion* sp. *Fungal Biol* **124**: 102–109.

Herrero, N. (2016) A novel monopartite dsRNA virus isolated from the entomopathogenic and nematophagous fungus *Purpureocillium lilacinum*. *Arch Virol* **161**: 3375–3384.

Hong, Y., Dover, S.L., Cole, T.E., Brasier, C.M., and Buck, K.W. (1999) Multiple mitochondrial viruses in an

isolate of the Dutch Elm disease fungus *Ophiostoma novo-ulmi*. *Virology* **258**: 118–127.

Jo, Y., Choi, H., Kim, S-M, Kim, S-L, Lee, B. C., and Cho, W. K. (2017) The pepper virome: natural co-infection of diverse viruses and their quasispecies. *BMC Genomics* **18**: 453. http://doi.org/10.1186/s12864-017-3838-8.

Jung, T., Perez-Sierra, A., Durán, A., Horta Jung, M., Balci, Y., and Scanu, B. (2018) Canker and decline diseases caused by soil- and airborne *Phytophthora* species in forests and woodlands. *Persoonia* **40**: 182–220.

Kashif, M., Jurvansuu, J., Vainio, E.J., and Hantula, J. (2019) Alphapartitiviruses of *Heterobasidion* wood decay fungi affect each other's transmission and host growth. *Front Cell Infect Microbiol* **9**: 64.

King, A.M., Adams, M.J., Lefkowitz, E.J., and Carstens, E.B. (2011) *Virus Taxonomy: IXth Report of the International Committee on Taxonomy of Viruses*, Vol. **9**. Waltham, MA, USA: Elsevier, Academic Press.

Kozlakidis, Z., Brown, N.A., Jamal, A., Phoon, X., and Coutts, R.H.A. (2010) Incidence of endornaviruses in *Phytophthora* taxon douglasfir and *Phytophthora ramorum*. *Virus Genes* **40**: 130–134.

Kumpula, R., Tuovinen, P., and Känninen, J. (2020) *Selvitys viljeltyjen marjakasvien taimiaineistosta*. *Marjaosaamiskeskus/Savogrow*. *63 Degrees North - Pohjoisen erikoiskasvit euroiksi*, *project report*. URL https://haapavesi-siikalatva.fi/wp-content/uploads/2020/09/Selvitys-viljeltyjen-marjakasvien-taimiaineistosta.pdf (in Finnish).

Lee, S.H., Yun, S.-H., Chun, J., and Kim, D.-H. (2017) Characterization of a novel dsRNA mycovirus of *Trichoderma atroviride* NFCF028. *Arch Virol* **162**: 1073–1077.

Lilja, A., Karjalainen, R., Parikka, P., Kammiovirta, K., and Nuorteva, H. (1998) Pathogenicity and genetic variation of *Phytophthora cactorum* from silver birch and strawberry. *Eur J Plant Patholol* **104**: 529–535.

Lin, Y.-H., Fujita, M., Chiba, S., Hyodo, K., Andika, I.B., Suzuki, N., and Kondo, H. (2019) Two novel fungal negative-strand RNA viruses related to mymonaviruses and phenuiviruses in the shiitake mushroom (*Lentinula edodes*). *Virology* **533**: 125–136.

Liu, H., Fu, Y., Xie, J., Cheng, J., Ghabrial, S.A., Li, G., *et al*. (2012) Evolutionary genomics of mycovirus-related dsRNA viruses reveals cross-family horizontal gene transfer and evolution of diverse viral lineages. *BMC Evol Biol* **12**: 91.

Moriyama, H., Horiuchi, H., Nitta, T., and Fukuhara, T. (1999) Unusual inheritance of evolutionarily-related double-stranded RNAs in interspecific hybrid between rice plants *Oryza sativa* and *Oryza rufipogon*. *Plant Mol Biol* **39**: 1127–1136.

Nellist, C.F., Vickerstaff, R.J., Sobczyk, M.K., Marina-Montes, C., Wilson, F.M., Simpson, D.W., *et al*. (2019) Quantitative trait loci controlling *Phytophthora cactorum* resistance in the cultivated octoploid strawberry (*Fragaria* × *ananassa*). *Hortic Res* **6**: 60.

Nerva, L., Forgia, M., Ciuffo, M., Chitarra, W., Chiapello, M., Vallino, M., *et al*. (2019) The mycovirome of a fungal collection from the sea cucumber *Holothuria polii*. *Virus Res* **273**: 197737.

Nerva, L., Varese, G.C., and Turina, M. (2018) Different approaches to discover mycovirus associated to marine organisms. In *Viral Metagenomics. Methods in Molecular Biology*, Vol. **1746**, Pantaleo, V., and Chiumenti, M. (eds). New York, NY: Humana Press.

Ong, J.W.L., Li, H., Sivasithamparam, K., Dixon, K.W., Jones, M.G.K., and Wylie, S.J. (2016) Novel Endorna-like viruses, including three with two open reading frames, challenge the membership criteria and taxonomy of the *Endornaviridae*. *Virology* **499**: 203–211.

Ong, J.W.L., Sivasithamparam, K., Dixon, K.W., Jones, M.G.K., and Wylie, S.J. (2017) The challenges of using high-throughput sequencing to track multiple bipartite mycoviruses of wild orchid-fungus partnerships over consecutive years. *Virology* **510**: 279–304.

Poimala, A., and Vainio, E. (2020) Complete genome sequence of a novel toti-like virus from the plant pathogenic oomycete *Phytophthora cactorum*. *Arch Virol* **165**: 1679–1682.

Sasai, S., Tamura, K., Tojo, M., Herrero, M.L., Hoshino, T., Ohki, S.T., and Mochizuki, T. (2018) A novel non-segmented double-stranded RNA virus from an Arctic isolate of *Pythium polare*. *Virology* **522**: 234–243.

Shi, M., Neville, P., Nicholson, J., Eden, J.S., Imrie, A., and Holmes, E.C. (2017) High-resolution metatranscriptomics reveals the ecological dynamics of mosquito-associated RNA viruses in Western Australia. *J Virol* **91**: e00680–e00617.

Shiba, K., Hatta, C., Sasai, S., Tojo, M., Ohki, S.T., and Mochizuki, T. (2018) Genome sequence of a novel partitivirus identified from the oomycete Pythium Nunn. *Arch Virol* **163**: 2561–2563.

Shiba, K., Hatta, C., Sasai, S., Tojo, M., Ohki, S.T., and Mochizuki, T. (2019) A novel toti-like virus from a plant pathogenic oomycete *Globisporangium splendens*. *Virology* **537**: 165–171.

Simmonds, P., Adams, M., Benkő, M., Breitbart, M.M., Brister, J.R., Carstens, E.B., *et al*. (2017) Virus taxonomy in the age of metagenomics. *Nat Rev Microbiol* **15**: 161–168.

Stensvand, A., Herrero, M.L., and Talgo, V. (1999) Crown rot caused by *Phytophthora cactorum* in Norwegian strawberry production. *EPPO Bull* **29**: 155–158.

Vainio, E.J., Keriö, S., and Hantula, J. (2011) Description of a new putative virus infecting the conifer pathogenic fungus *Heterobasidion parviporum* with resemblance to *Heterobasidion annosum* P-type partitivirus. *Arch Virol* **156**: 79–86.

Vainio, E.J., Piri, T., and Hantula, J. (2013) Virus community dynamics in the conifer pathogenic fungus *Heterobasidion parviporum* following an artificial introduction of a partitivirus. *Microb Ecol* **65**: 28–38.

Valverde, R.A., Khalifa, M.E., Okada, R., Fukuhara, T., Sabanadzovic, S., and Consortium, I.R. (2019) ICTV virus taxonomy profile: Endornaviridae. *J Gen Virol* **100**: 1204–1205.

Velasco, L., Arjona-Girona, I., Cretazzo, E., and Lopez-Herrera, C. (2019) Viromes in *Xylariaceae* fungi infecting avocado in Spain. *Virology* **532**: 11–21.

Wilcox, W.F. (1989) First report of crown rot (*Phytophthora cactorum*) of strawberry in eastern North America. *Plant Dis* **73**: 183.

Wu, S., Cheng, J., Fu, Y., Chen, T., Jiang, D., Ghabrial, S. A., and Xie, J. (2017) Virus-mediated suppression of host

non-self recognition facilitates horizontal transmission of heterologous viruses. *PLoS Pathog* **13**: e1006234.

Yang, G., Hu, F., Shi, N., Wang, P., and Huang, B. (2020) A novel non-segmented double-stranded RNA virus isolated from the basal fungus *Conidiobolus* sp. *Arch Virol* **165**: 1919–1923.

Zhang, R., Hisano, S., Tani, A., Kondo, H., Kanematsu, S., and Suzuki, N. (2016) A capsidless ssRNA virus hosted by an unrelated dsRNA virus. *Nat Microbiol* **1**: 15001.

Zhang, T., Zeng, X., Zeng, Z., Hu, Z., and Yang, F. (2018) A novel monopartite dsRNA virus isolated from the phytopathogenic fungus *Ustilaginoidea virens* strain GZ-2. *Arch Virol* **163**: 3427–3431.

Zhou, Q., Zhong, J., Hu, Y., and Da Gao, B. (2016) A novel nonsegmented double-stranded RNA mycovirus identifed in the phytopathogenic fungus *Nigrospora oryzae* shows similarity to partitivirus-like viruses. *Arch Virol* **161**: 229–232.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supplementary file S1. Complete polyprotein sequences of Phytophthora cactorum alphaendornavirus 1 (PcAEV1), Phytophthora cactorum alphaendornavirus 2 (PcAEV2), Phytophthora cactorum alphaendornavirus 3 (PcAEV3), and RdRp of Phytophthora cactorum bunya-like virus 2 (PcBV2).

Supplementary Table S1. The screening primer pairs used in the identification of virus-infected isolates.

Supplementary Table S2. The locations of double peaks in the near-complete genome Sanger sequences and/or polymorphic sites in mapped RNA-seq reads.

Supplementary Table S3. Viruses and their variants hosted by the *Phytophthora cactorum* isolates of this study. The isolation year, location and the strawberry cultivar is shown, if known.