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REVIEW PAPER

Evolutionary forces at work in partitiviruses

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Abstract

The family *Partitiviridae* consists of dsRNA viruses with genome separated into two segments and encoding replicase and capsid protein only. We examined the nucleotide diversity expressed as the ratio d_N/d_S of nonsynonymous and synonymous substitutions, which has been calculated for 12 representative viruses of all five genera of partitiviruses. We can state that strong purifying selection works on both the RdRp and CP genes and propose that putative positive selection occurs also on the RdRp genes in two viruses. Among the 95 evaluated viruses, wherein both segments had been sequenced, 8 viruses in betapartitiviruses and 9 in alphapartitiviruses were identified as reassortment candidates because they differ extremely in their CP identity even as they are related in terms of RdRp. Furthermore, there are indications that reassortants are present among isolates of different viruses.

Keywords Capsid protein · Negative/positive selection · Reassortment · RNA polymerase · Symbiosis

Introduction

The family *Partitiviridae* consists of a rapidly broadening group of relatively simple viruses present in plants, fungi, and protozoa. The dsRNA genome of partitiviruses is about 3–5 kbp in length and is composed of two segments, each possessing a single ORF and occasionally associated with satellite RNAs or defective RNAs [1]. One segment encoding capsid protein (CP) and the other encoding replicase (RdRp) are encapsidated separately in isometric particles 35–42 nm in diameter [2, 3]. The partitiviruses are transmitted vertically via spores and seeds of their hosts and horizontally via hyphal anastomosis and during cell division. Most probably, partitiviruses do not have extracellular routes for infection [4]. The plant-infecting partitiviruses are classified now in Alpha-, Beta-, and Deltapartitivirus genera, while the fungal viruses are present in Alpha-, Beta-, and Gammapartitivirus genera. The genus Cryspovirus has a single member infecting protists. Host preference does not, however, constitute a strict border: The Penicillium aurantiogriseum partitivirus 1, for example, can replicate in *Nicotiana benthamiana* and in *N. tabacum* protoplasts as well as in its original fungal host [5]. There presently are 60 species classified within this family: 14 species in the genus Alphapartitivirus, 17 species in Betapartitivirus, 8 species in Gammapartitivirus, 5 species in Deltapartitivirus, and 1 species in the genus Cryspovirus. Fifteen unassigned species are mentioned in the 2017 release of the International Committee on Taxonomy of Viruses (ICTV), and more than 130 names of unclassified partitiviruses are present in GenBank (Nov 2018).

In addition to free-living partitiviruses, many endogenous sequences homologous to CP or RdRp of partitiviruses have been found in plants, fungi, arthropods, protozoa, nematodes, gastropods, tunicates, insects, and fish [4, 6]. They could be classified into the existing partitivirus genera, and the endogenous sequences cluster together with the CP and RdRp of the respective partitivirus [7]. Recently, a new partitivirus of unknown origin but close to fungus-infecting partitiviruses was detected in human sera [8], thus indicating that the hosts of the viruses may include animals as well. We can expect that free-living partitiviruses infecting animals will be discovered in future.

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Inasmuch as the number of novel partitivirus descriptions increases with every passing month, there exists ample evidence that the concept of a very simple virus with only two genes (the replicase and the capsid protein), each placed on a separate segment, is highly successful in evolution and is very capable in using plant, fungal, and animal cells for its expression and maintenance within the environment. None of the presently known partitiviruses cause acute disease in their hosts. Plant-infecting partitiviruses have been previously classified as cryptoviruses to describe their presence without causing detrimental effect to their hosts [2]. The fungi-inhabiting partitiviruses only rarely reduce the growth rate of their fungal hosts or change their pigmentation [9–12]. Aspergillus fumigatus partitivirus 1 could be an exception, as its infection has been shown to result in an abnormal phenotype consisting of aconidial sectors, change of pigmentation, and lower host fitness as measured by growth rate and hyphal biomass in broth [13]. Recent data have shown, too, that in two-step interactions the Talaromyces marneffei partitivirus 1 (a putative gammapartitivirus) was found to enhance the virulence of the dimorphic fungus *T. marneffei* in mice, causing shortened survival time and higher fungal burden in the mice's organs [14]. Partitiviral infections are most often characterized as persistent infections during which the virus remains in the cells of infected individuals and involving stages of both silent and productive infection without rapidly killing or damaging the host cells. In a few cases, wherein the virus is present in all fungal samples evaluated, the infection might be assumed to be symbiotic, although we do not know what benefit the virus may be providing to the fungal host. Such is the case of Curvularia partitivirus 1 and Cryptosporidium parvum partitivirus [15, 16], which could be used as tracers for the hosts. Synergistic effect has been described also for Lentinula edodes virus—HKB (putative Phlegivirus) and Lentinula edodes partitivirus 1, as infection with the phlegivirus is associated with the increasing the accumulation of the RdRp segment of the partitivirus [17]. The hypovirus phenomenon (highly interesting for its putative use for biocontrol of the pathogen), wherein the virus debilitates the pathogenic effect of its fungal host on plants, is not common for partitiviruses but has been described for Rhizoctonia solani partitivirus 2 (an alphapartitivirus) [11] and for Sclerotinia sclerotiorum partitivirus 1 (a betapartitivirus) [9].

The diversity of mycoviruses was unknown or underestimated for many years. Surprisingly, few partitiviruses were sequenced from different isolates and locations. The majority of them have been described and sequenced only from single host species, as it is believed that mycoviruses in general have narrow host ranges in nature and abilities only to infect the same or closely related natural vegetative compatibility groups of the same fungal species [5]. Nevertheless, some partitiviruses have been transferred experimentally to different hosts in the laboratory, thereby proving their ability to colonize a species other than that from which they were first isolated. Sclerotinia sclerotiorum partitivirus 1 was co-transmitted via hyphal tips to *Sclerotinia nivalis* and *Botrytis cinerea* [9]. Heterobasidion RNA virus 3 from *Heterobasidion ecrustosum* was transmitted via hyphal anastomosis to *H. abietinum* and *H. occidentale* and the infection was stable there [18]. Protoplasts of *Cryphonectria parasitica* and *Glomerella cingulata* were inoculated with Rosellinia necatrix partitivirus 1 and horizontal transmission from the newly infected strains to virus-free strains was readily achieved by dual culture and hyphal anastomosis [18]. No sequence analyses of viruses from these novel hosts have been performed to screen whether novel virus sequence variants occur or were selected.

High sequence identity has been observed in the few population studies of partitiviruses. The 21 sequences of Ustilaginoidea virens virus 1 strain GX-1 showed 91.1–100% identity at the nucleotide level and 91.8–100% at the amino acid level despite that the isolates were obtained from different geographical locations in China [19]. In another study of partitivirus infecting *Pseudogymnoascus destructans* fungus joined with lethal disease in bats, in which more than 40 virus isolates were sequenced, the RdRp identity was in the range 99.7–99.9% and the CP identity was in the range 96.8–98.4% [20]. Evaluation of the nucleotide sequence variability of the orchid partitiviruses revealed that CP and RdRp segments are subject to differential rates of evolutionary change. Shorter branch lengths in the RdRp-generated phylogeny suggest that partitivirus RdRps evolve at slower rates than do CPs [21]. Botella et al. [22] calculated the selection pressure parameters for CP and RdRp coding regions of 46 isolates of Gremmeniella abietina virus MS1 and stated that active purifying selection exists on these sequences.

As viruses having separately encapsidated genome segments, the reassortant phenomenon observed in plant ssRNA cucumo-, polero-, umbraviruses and comoviruses [23–25], ssDNA geminiviruses [26], and dsRNA mycoreoviruses [27] could be expected to occur also in partitiviruses. The prerequisites for such recombination exist, as mixed infections of fungi-infecting partitiviruses occur relatively often. Betapartitivirus and alphapartitivirus have been described in a single strain of *Helicobasidium mompa* [28]. Penicillium stoloniferum viruses F and S (both gammapartitiviruses) have been shown to co-infect a *Penicillium stoloniferum* strain, and their coincident expression has been demonstrated in the host [29, 30]. Sodiomyces alkalinus partitivirus 1 (a betapartitivirus) and Colletotrichum truncatum partitivirus 1 strain SAL (a gammapartitivirus) were described in the alkalophilic fungus *Sodiomyces alkalinus* [31]. Recently, an extremely complex mixture of 16 different alpha- and betapartitiviruses was

detected in two isolates of mycorrhizal *Ceratobasidium* fungus associated with *Pterostylis* orchids [21]. Putative reassortants have rarely been recognized, however. Most often they have been documented in cases when the phylogenetic trees, derived either from CP or RdRp sequences' evaluation, differ and the given virus clusters are in different clades or with different neighbour species. One hint that reassortment can indeed occur among closely related partitiviruses is based on data of Vainio et al. [32] suggesting that dsRNA1 (RdRp) of the betapartitivirus Heterobasidion partitivirus 8 came from an ancestor more closely related to Fusarium poae virus 1, while its dsRNA2 (CP) came from one more closely related to Pleurotus ostreatus virus 1. Comparison of phylogenetic trees separately constructed from either RdRp or CP sequences reveals that none of the partitiviruses had undergone genome-segment reassortment between genera [2].

In this paper, we evaluate sequence data of selected partitiviruses and calculate selection pressure. We searched for putative reassortants among all available sequenced partitiviruses.

Global selection pressure

It is assumed that mycoviruses strongly depend on their host fungi for dispersal, as they have no extracellular stage and have developed in accordance with their hosts. In fact, only virus isolates originating from single host species have been evaluated to date. It is hypothesized that Gremmeniella abietina RNA virus 6 (a gammapartitivirus) evolved with its fungal host. This hypothesis is supported by the fact that only asexual reproduction has been reported in the Spanish population of the fungus [22]. Very low virus diversity has been observed in 32 isolates of Heterobasidion partitivirus 2 (a betapartitivirus) and Heterobasidion partitivirus 6, where 9.3×10^{-4} mutations per nt and 5.5×10^{-4} to 2.2×10^{-3} mutations per nt, respectively, were observed [33]. For calculating nucleotide diversity, the coding sequences for CP and RdRp were obtained from at least four different isolates per species retrieved from GenBank (Table 1). Replicase and CP genes (where available) from isolates of Vicia cryptic virus, Heterobasidion partitivirus 13, Heterobasidion partitivirus 20 (all alphapartitiviruses), Cucurbitaria piceae partitivirus 1, Heterobasidion partitivirus 2, Heterobasidion

Table 1 Viruses and sequences used in calculation of evolutionary forces

	n	nt	GenBank AC No for RdRp	*n*	nt	GenBank AC No. for CP
Alphapartitiviruses						
Vicia cryptic virus	6	1848	EF173396, EF173389, AY751737, EF173392, EF173394, EU605883	6	1461	EF173390-1,EF173393, EF173395, EU605884, NC_007242
Heterobasidion partitivirus 13	4	984	KF963177, KF963183, KF963179, KF963181	4	732	KF963178, KF963180, KF963182, KF963184
Heterobasidion partitivirus 20	10	612	KY911256, KY911258-66,	10	354	KY911257, KY911267-74
Betapartitiviruses						
Cucurbitaria piceae partitivirus1	7	2121	KX244820,-4,-6,-8, KT343866, MK117740-1	7	1986	KX244821 -3, -5, -7, -9, KT343867, MK117739
Heterobasidion partitivirus 2	7	2148	HM565953, KF551880-5	–	–	–
Heterobasidion partitivirus 7	4	2154	JN606091, KF551886-7, KY859973	–	–	–
Gammapartitiviruses						
Gremmeniella abietina RNA virus MS1	45	513	KJ786323-66	46	558	AY089994, KJ786367-410
Pseudogymnoascus destructans virus	47	927	KY207498-543, KP128044	45	1086	KY207453-97
Deltapartitiviruses						
Fig cryptic virus	7	1350	FR776004-9, FR687854	–	–	–
Pepper cryptic virus 2	6	1149	KX525268, KX905077, LC195294, LC325817, JN117278, KY923703	7	1290	LC325818, JN117279, KX905078, LC195295, KT931616, KR676354
Cryspovirus						
Cryptosporidium parvum virus 1	26	1449	LC014992-15015, KY884720, CPU95995	24	897	CPU95996, KY884721, LC085158-79
Non-classified						
Ustilaginoidea virens nonsegmented V1	13	555	KJ605398-410	–	–	–

n Number of different sequences, *nt* length of sequences

partitivirus 7 (all betapartitiviruses), Gremmeniella abietina virus, Pseudogymnoascus destructans virus (gammapartitiviruses), Fig cryptic virus, Pepper cryptic virus 2 (deltapartitiviruses), Ustilaginoidea virens nonsegmented virus 1 (an unclassified partitivirus) and Cryptosporidium parvum virus 1 (a cryspovirus) were CLUSTALW aligned in MEGA7 [34], inspected manually, and selection pressure on the protein-coding regions was assessed based on ratio (d_N/d_S), of nonsynonymous to synonymous nucleotide substitutions (Table 2). These assessments were made using DnaSP v5 software [35] according to the Nei-Gojobori model and with Jukes and Cantor correction [36]. Sites with alignment gaps or missing data were omitted from the analysis. In the case of RdRp analysis of Heterobasidion partitivirus 2, only the nonsynonymous substitutions were observed.

In all viruses but Heterobasidion partitivirus 20, the d_N/d_S of RdRp is higher than that for CP, which means that the CP is under stronger selection pressure in these species (Table 2). Viruses from all five genera have d_N/d_S nucleotide diversity values < 1, which points to strong negative selection of the given genes. Very low values for RdRp in Heterobasidion partitivirus 7 and Ustilaginoidea virens nonsegmented virus 1 and for CP in Heterobasidion partitivirus 13 and Cryptosporidium parvum virus 1 indicate homogeneity

Table 2 Global selection pressure on polymerase and capsid protein genes of selected partitiviruses

	RdRp d_N/d_S	CP d_N/d_S
Alphapartitivirus		
Vicia cryptic virus	0.134	0.057
Heterobasidion partitivirus 13	0.058	0.005
Heterobasidion partitivirus 20	0.046	0.160
Betapartitivirus		
Cucurbitaria piceae virus	0.243	0.163
Heterobasidion partitivirus 2	*	na
Heterobasidion partitivirus 7	0.015	na
Gammapartitivirus		
Gremmeniella abietina RNA virus MS1	0.082	0.059
Pseudogymnoascus destructans virus	1.284	0.571
Deltapartitivirus		
Fig cryptic virus	1.096	na
Pepper cryptic virus 2	0.157	0.124
Unclassified		
Ustilaginoidea virens nonsegmented V1	0.018	na
Cryspovirus		
Cryptosporidium parvum virus 1	0.030	0.013

Nucleotide diversity parameter d_N/d_S was calculated with Jukes and Cantor correction

na Not available

*Estimated d_N/d_S was infinite due to lack of synonymous substitutions ($d_S = 0$)

to identity of the viral populations in the given genes. It should be noted that each of the given isolates originated from only a single host species (e.g. Heterobasidion partitivirus 20 originated from *H. annosum* fungus, Fig cryptic virus from *Ficus carica* plants). One can speculate that the very low values d_N/d_S in *Heterobasidion* viruses calculated in this work may be related to the fact of sample collection on a limited area within a single forest, where the host (*Heterobasidion* fungus) could represent identical or closely related vegetative clones [33].

On the other hand, positive selection of mycoviruses is occasionally reported. Feau et al. [37] reported sites under positive selection in both ORFs of Cryphonectria hypovirus 1. In the case of the replicase gene of partitiviruses, there are two exceptions: Those d_N/d_S values for Fig cryptic virus and Pseudogymnoascus destructans virus were > 1, and this could indicate positive selection of these two genes. We ran aBSREL, RELAX, FUBAR and MEME tests available at the Datamonkey website (http://www.datamonkey.org) for detection of diversifying selection in these two genes. FUBAR identified only one site (amino acid 237) in Pseudogymnoascus destructans virus under positive selection, but the other tests did not support this finding. Five and four sites (amino acid positions 44, 52, 197, 236, and 396 and 121, 169, 197, and 236) were identified with FUBAR and MEME tests, respectively, for Fig cryptic virus under positive selection, and aBSREL found evidence of episodic diversifying selection in the phylogeny of Fig cryptic virus isolates.

We conclude that the RdRp as well as CP genes of viruses from all partitivirus genera undergo purified selection in their hosts. We offer also some indications that RdRp genes of Fig cryptic virus and also of Pseudogymnoascus destructans virus revealed diversifying selection (positive selection), despite the fact that in both viruses, its CP segments revealed negative selection. Coincidentally, these are the only viruses the samples of which were taken from more divergent hosts (different plant cultivars in the case of Fig cryptic virus) or when they inhabit highly mobile hosts, as in the case of Pseudogymnoascus destructans virus.

Prediction of putative reassortants

In the cases of segmented viruses, reassortment of the genomic segment could also constitute a way for novel genotypes formation, thus providing the potential for viruses to evolve. Genome-segment reassortment has been shown to occur among members of other families of segmented dsRNA viruses, such as Reoviridae (9–12 genome segments), Cystoviridae (3 genome segments) and Birnaviridae (2 genome segments) [2]. In contrast to influenza virus A (family Orthomyxoviridae), which has all segments

encapsidated within a single particle [38], the reassortment of partitiviruses, which have separately encapsidated segments, could occur more frequently than is currently supposed.

For detecting putative reassortment, 95 approved and not yet approved partitiviruses wherein both segments had been sequenced were retrieved from GenBank and used for evaluation. The CP and RdRp protein sequences were CLUSTALW aligned, then Pol/Pol and CP/CP matrices of mutual sequence identities were calculated. Such matrices were calculated also for the distinct genera. Pairs of viruses for which the Pol and CP identities greatly differ should be marked here as reassortment candidates and were evaluated in detail.

Inasmuch as both segments forming the partitivirus are necessary for virus expression and are present in the host organism, we can expect that they evolve simultaneously. Furthermore, if the segments have constituted the virus for a long time, we can expect that the mutual differences in RdRp sequence identity and in CP sequence identity will correlate. Large differences in closely related viruses could indicate that the first and second segments evolved in different conditions (perhaps in different hosts) and that the present virus could be a reassortant more similar to one virus in its RdRp sequence and to another virus in its CP sequence. On the pairwise comparison plots in Figs. 2, 3, 4, 6 such virus is marked as a point situated more distantly from the observed correlation (distant black points) and the RdRp − CP subtraction of identity values (red points in the graph) are higher than is typical for related viruses. For all genera, we inspected all pairs of viruses where the RdRp − CP value was > 40%. In cases when the CP sequences were more related than were the RdRp sequences, the RdRp − CP value was < 0.

Despite that CP of partitiviruses in distinct hosts undergo stronger purifying selection than does the RdRp gene, as shown above, the alignment of RdRp of different viruses shows greater conservation of the RNA polymerase motives among species than is the conservation of motives in the CP gene [2]. Calculated were a 95 × 95 matrix of pairwise identities in the RdRp gene and an identical matrix of pairwise identities in the CP gene (Fig. 1). Strong correlation was found between the RdRp and CP distances: Viruses closely related in their RdRp genes were also revealed to have high identity in their CP genes, and exceptions to this were observed only rarely. In our analyses, the CP identities of distinct viruses were often half those of the RdRp.

For more detailed examinations, we aligned RdRp and CP and calculated matrices for distinct genera, where putative species also were included (see Supplementary Table 1 for the species). Ten viruses were evaluated as **gammapartitiviruses** and 17 viruses as **deltapartitiviruses**. The RdRp and CP matrices were subtracted and plotted to visualize the differences (red points in Figs. 2, 3, 4, 6). In gammapartitiviruses and deltapartitiviruses, strong correlation ($R^2 = 0.945$ and 0.858, respectively) in RdRp and CP identities was observed (Figs. 2, 3). The RdRp − CP identities were < 20% (16.5 ± 1.6 with 95% confidence) and < 30% (26.8 ± 0.8 with 95% confidence), respectively, in these genera. In gammapartitiviruses, Gremmeniella abietina RNA viruses MS1

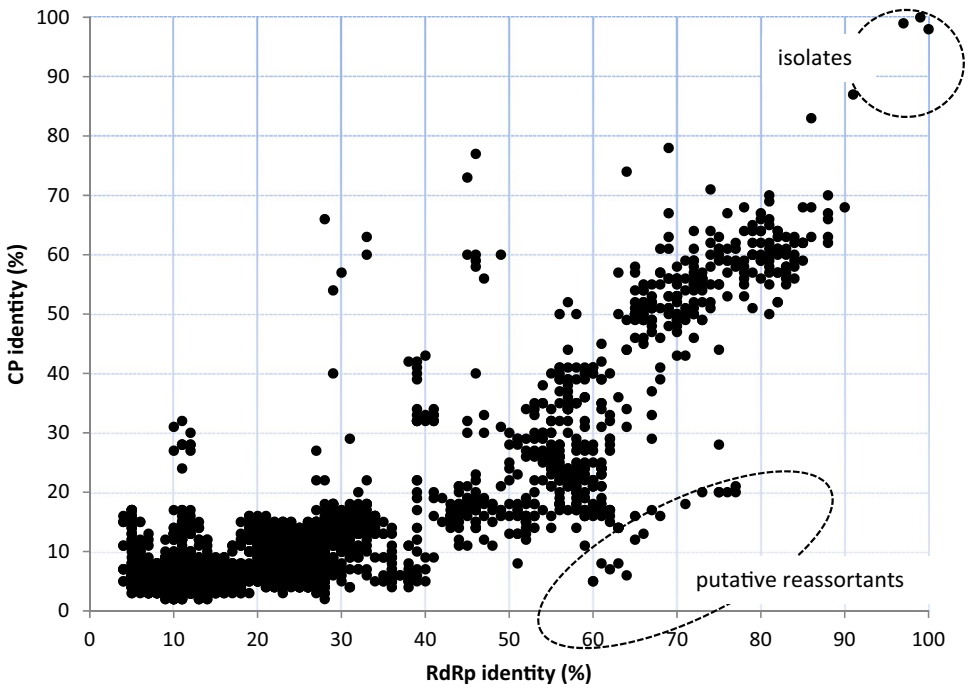

Fig. 1 Pairwise comparison between RdRp sequences plotted against CP comparisons for the same pairs of sequences from 95 partitiviruses. Each point represents a relationship between two viruses in their RdRp and CP amino acid sequences. Those points representing isolates of distinct viruses are circled. Points representing pairs of viruses strongly related in one gene but weakly related in the other gene, and therefore representing putative reassortants are identified

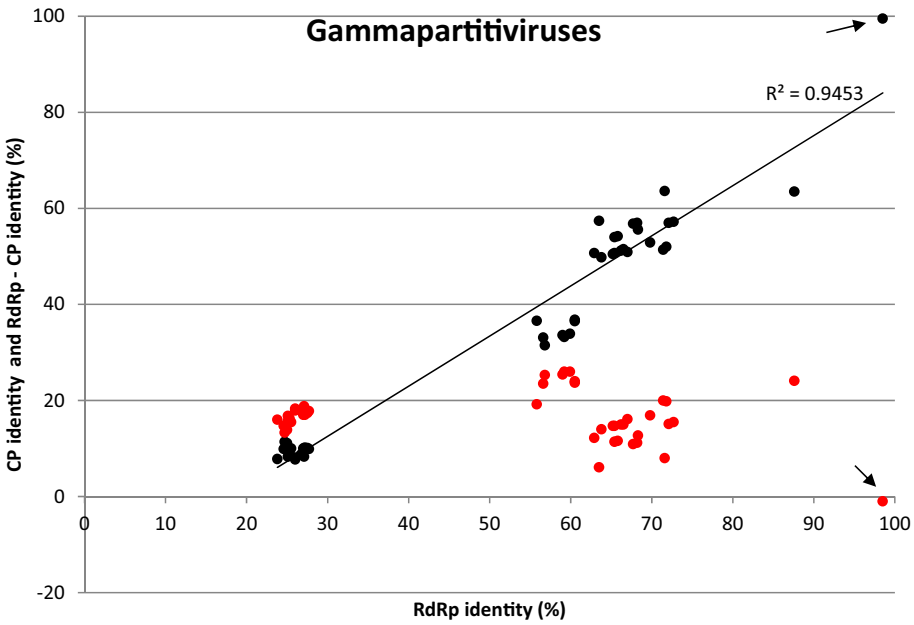

Fig. 2 Pairwise comparison between RdRp sequences plotted against CP comparisons of the same pairs of gammapartitiviruses (black dots) and against RdRp identity minus CP identity (red dots). The point representing Gremmeniella abietina MS1 and MS2 viruses is indicated by an arrow

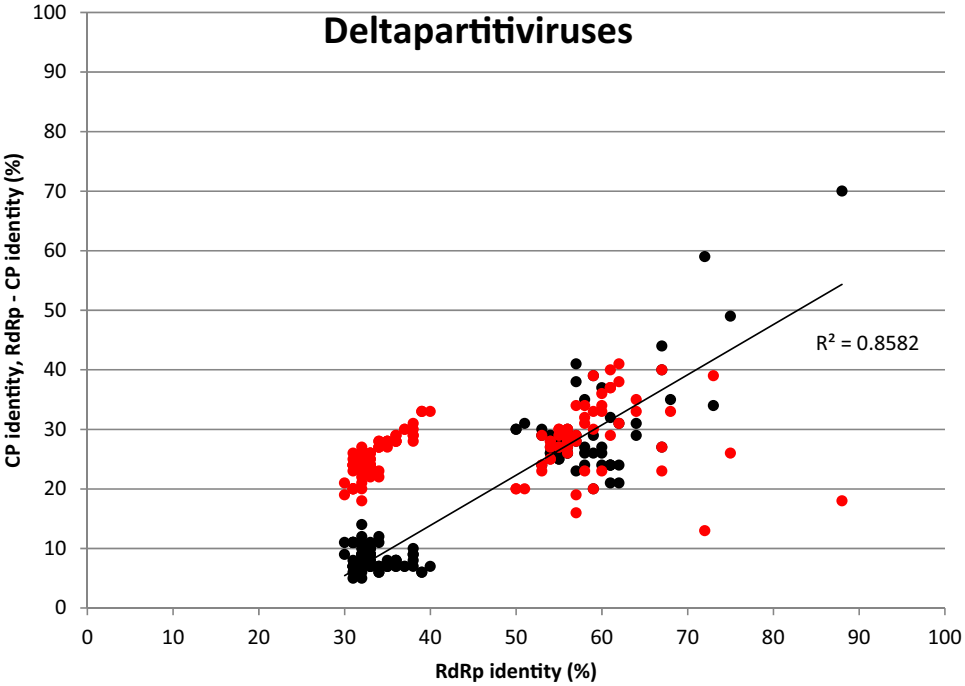

Fig. 3 Pairwise comparison between RdRp sequences plotted against CP comparisons of the same pairs of deltapartitiviruses (black dots) and against RdRp identity minus CP identity (red dots)

and MS2 more probably represent strains of a single virus species that are 98.5% and 99.5% identical in RdRp and CP, respectively (Fig. 2). No putative reassortants were proposed in these genera.

In evaluating **betapartitiviruses**, we compared 25 viruses (See Supplementary Table 1). In this genus, too, the RdRp and CP identities are strongly correlated ($R^2 = 0.733$). Pleurotus ostreatus virus 1 and Heterobasidion partitivirus 8 comprise here the only pair of viruses for which the CP genes are more identical to one another than are the RdRp genes (78% vs. 58%) (Fig. 4). This is quite a different level of similarity than is seen in the rest of the viruses, and most probably these viruses are relatively novel reassortants with different RdRp segments. It should be noted that there also are viruses among the betapartitiviruses sharing significant similarity in RdRp but whose CP genes are only very distantly related with 20–30% aa identity. They are Rosellinia necatrix partitivirus 3,

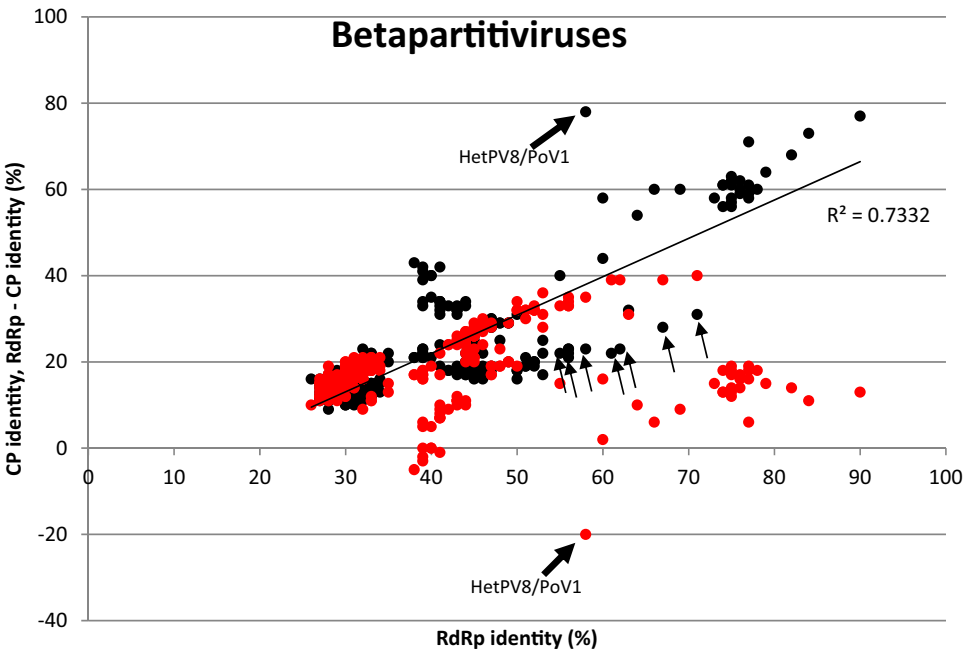

Fig. 4 Pairwise comparison between RdRp sequences plotted against CP comparisons of the same pairs of betapartitiviruses (black dots) and against RdRp identity minus CP identity (red dots). The points representing Heterobasidion partitivirus 8 (HetPV8) and Pleurotus ostreatus virus 1 (PoV1) reassortants are marked with bold arrows. Points representing putative reassortants are marked with thin arrows

Sclerotinia sclerotiorum virus 1, Cucurbitaria piceae partitivirus 1, and Fusarium poae virus 2 (Fig. 5). All these viruses could be presumed to be putative reassortants. Furthermore, there are viruses, like Rosellinia necatrix partitivirus 6 and 1-W8 and Podosphaera prunicola partitivirus 4, whose RdRp identities with other viruses are less prominent even as their CP genes are unrelated to any other known CP. These viruses also could be candidates for putative reassortants.

Among the **alphapartitiviruses,** we evaluated 42 viruses for which complete ORFs of both RdRp and CP were known. Members of this genus differ greatly in their CP gene sequences and a phylogeny tree derived from CP has been shown not to correlate with the RdRp tree [39].

Within this genus, similarly as among the betapartitiviruses, we identified one pair of viruses (Heterobasidion partitivirus 3 and Heterobasidion partitivirus 12) which have CP genes more identical than their RdRp genes (73% and

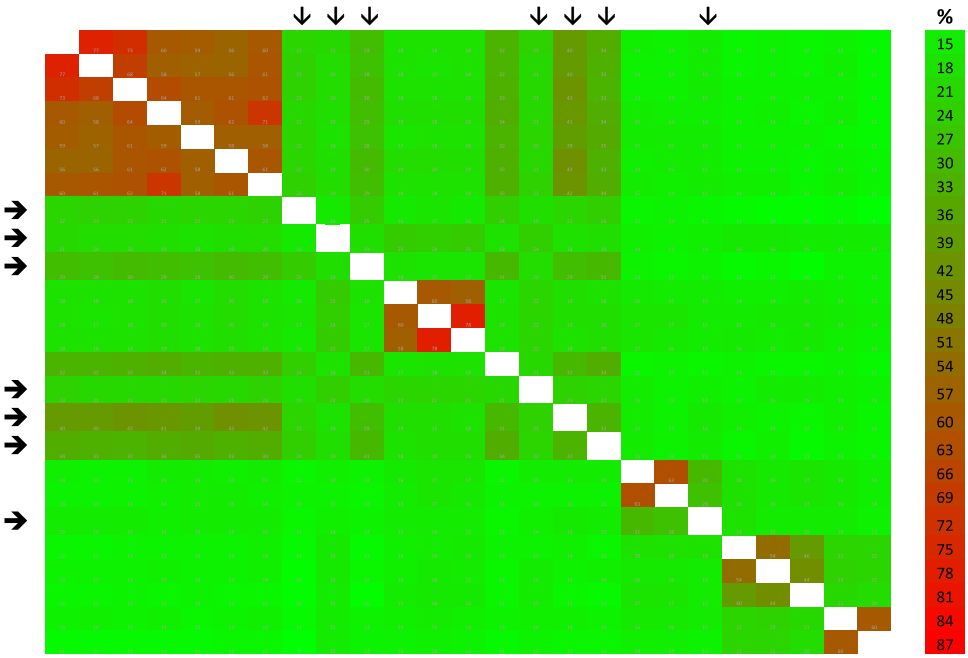

Fig. 5 Heat map of CP/CP identity of betapartitiviruses. Viruses with unique CP segments are marked with arrows

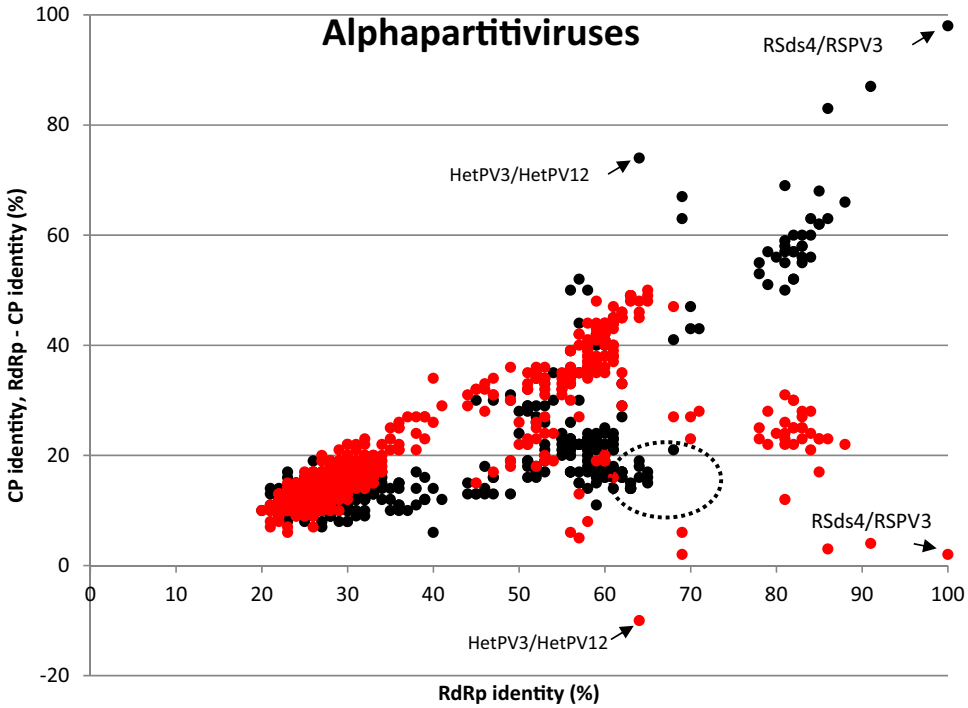

Fig. 6 Pairwise comparison between RdRp sequences plotted against CP comparisons of the same pairs of alphapartitiviruses (black dots) and against RdRp identity minus CP identity (red dots). The points representing putative reassortants Heterobasidion partitivirus 3 (HetPV3) and 12 (HetPV12) are marked with bold arrows. Points representing the mutual identities of putative reassortants Rhizoctonia solani dsRNA virus 3, Rhizoctonia solani virus 4, Rosellinia necatrix virus 9, as well as Heterobasidion partitiviruses 3, 12, 13, 15 and 20 are marked with a dotted circle. Rhizoctonia solani dsRNA virus 4 (RSds4) and Rhizoctonia solani partitivirus 3 (RSPV3) should be regarded as isolates

64%, respectively) (Fig. 6). Most probably, these viruses are relatively novel reassortants with different RdRp segments. Rhizoctonia solani dsRNA virus 4 and Rhizoctonia solani partitivirus 3 should be considered as isolates, as they are highly identical in both genes. As in betapartitiviruses, also in alphapartitiviruses there are viruses sharing high (> 60%) sequence identities in their RdRp genes but rather low (< 20%) identities in their CP genes. These are Rhizoctonia solani dsRNA virus 3, Rhizoctonia solani virus 4, Rosellinia necatrix virus 9, as well as Heterobasidion partitiviruses 3, 12, 13, 15, and 20 (Fig. S1). All these viruses could be presumed to be putative reassortants.

Despite the fact that mixed infections of alpha-, beta- and gammapartitiviruses have been described, and that mixed exposure is probably long enduring in hosts, some barriers to reassortment could also play their roles. Compartmentalization of viral replication and packaging to localized areas of the cell could be among those factors discouraging reassortment between genera. It has been shown that CP–CP dimerization for alphapartitiviruses occurs at the nuclear membrane, and for betapartitiviruses, it occurs close to cell walls within the cytoplasm [40]. There are no data, however, as to where replication occurs in gamma- and deltapartitiviruses. Usually, one or two molecules of RdRp are anchored to the interior capsid surface and the partitivirus particle is the place where RdRp mediates transcription of the encapsidated segment. The presence of conserved sequences functioning as a packaging/replication/identity signal has not, however, been identified for partitiviruses either generally or for the distinct genera. Short (3–6 nt) genus-specific motives only were observed close to the 5′-terminal "G". Also, conservative secondary structures close to the 5′-end are considered to be involved in RNA packaging and replication [41]. Erroneous identification of such signal (if it exists) could lead to acceptance of a different viral segment into the particle and the appearance of a reassortant. On the other hand, divergent members of the family Partitiviridae use strongly similar principles of capsid architecture, particle assembly, and RNA organization [42].

It is anticipated that most reassortment events yield viruses that are less fit than is either parent [43]. The reason for this is that viral components act in concert, but the enzymatic activity of partitiviral RdRp and CP with the host's environment is not known. For unknown reasons, viruses with highly different CP genes are more frequently detected. The opposite situation is rare, but it is documented here in the cases of Heterobasidion partitivirus 8/Pleurotus ostreatus virus and Heterobasidion partitivirus 3/Heterobasidion partitivirus 12 viruses. The betapartitivirus Heterobasidion partitivirus 8 may represent a reassortant in which the RdRp came from an ancestor more closely related to Fusarium poae partitivirus 1 and the CP from one more closely related to Pleurotus ostreatus virus 1 [2]. The reciprocal combination has not yet been found or was not viable (Fig. 7a). Heterobasidion partitivirus 3 and Heterobasidion partitivirus 12 more probably accepted identical CP segment from an unidentified virus and/or reservoir while lacking the original CP segments (Fig. 7b). This demonstrates that one CP could complement different RdRp segments. We did not expect that the integrated partitiviral genes could serve as a source of genes. The integrated viral

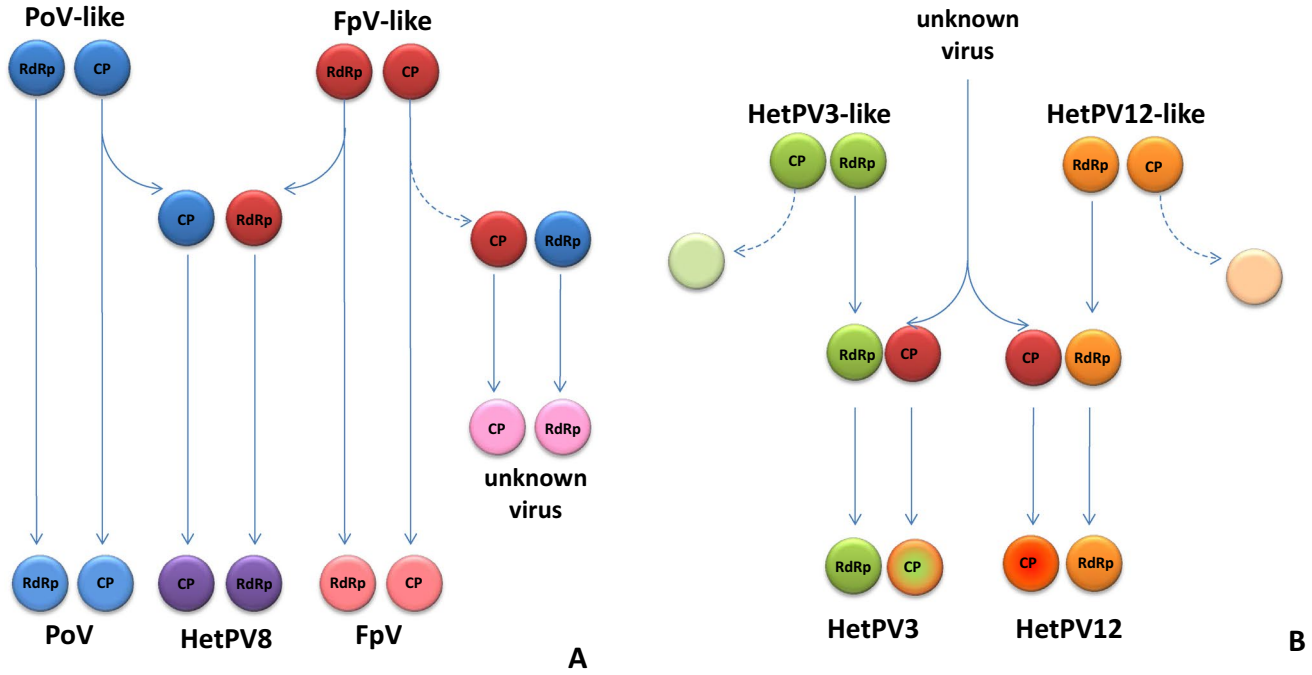

Fig. 7 **a** Model of Heterobasidion partitivirus 8 (HetPV8) creation by reassortment of CP segment from Pleurotus ostreatus-like virus (PoV-like) ancestor and the RdRp segment from Fusarium poae-like virus (FpV-like). **b** Model of Heterobasidion partitivirus 3 (HetPV3) and 12 (HetPV12) creations by reassortment of the CP segment from unknown ancestor with RdRp segments of Heterobasidion partitivirus 3-like (HetPV3-like) and 12-like (HetPV12-like) viruses

genes had apparently degenerated sequences, inasmuch as they contained frameshifts, internal stop codons, or deletions compared to viral genes. Some remained intact, however, and this implies that the viral genes (CP and/or RdRp) could play a role within their hosts when expressed. In some cases, phylogenetic analysis has indicated that endogenous sequences integration are ancestral and that horizontal transmission of these viruses had occurred between animals and fungi or between animals and plants [4, 6].

Last but not least, reassortment may exist between isolates of one species, since a number of gene variations arise during the host infection and are present in one cell [32, 33]. To test this hypothesis, we prepared concatenated CP-RdRp sequences of Vicia cryptic virus, Heterobasidion partitivirus 13 and 20, Cucurbitaria piceae partitivirus 1, Gremmeniella abietina RNA virus MS1, Pseudogymnoascus destructans virus, Pepper cryptic virus 2, and Cryptosporidium parvum virus 1 isolates, and run RDP4 analyses [44]. Recombination points between the CP and the RdRp genes were detected in 6 isolates of four different viruses (Table 3).

Summary

Comparison of the phylogenetic trees for reassortant analysis is insensitive to distantly related viruses, however. We calculated identity matrices and their subtraction for visualization of differences, but this method shows only extremes in RdRp and CP classification. We are unable to identify distantly related reassortant, even though such cases probably occur among alphapartitiviruses, until more sequence data will be available for comparison.

The nucleotide diversity expressed as the ratio d_N/d_S of nonsynonymous and synonymous substitutions has been calculated for representative viruses of all five genera of partitiviruses. We can state that strong purifying selection works on both the RdRp and CP genes and propose that putative positive selection occurs also on the RdRp genes in Fig cryptic deltapartitivirus and Pseudogymnoascus destructans gammapartitivirus. Among the 95 evaluated viruses, several other viruses were identified as

Table 3 Putative reassortants within distinct virus species

Reassortant isolate	*n*	Major parent Isolate	Minor parent Isolate	GENECONV *P* values	BootScan	MaxChi	Chimaera	SiScan	3Seq
Gremmeniella abietina RNA virus MS1	43								
MS1_29		MS1_8	MS1_41	–	–	5.5×10^{-5}	9.9×10^{-3}	6.0×10^{-9}	7.2×10^{-3}
MS1_33		MS1_24	MS1_36	–	–	4.3×10^{-3}	1.5×10^{-2}	–	2.2×10^{-2}
Pseudogymnoascus destructans virus	44								
M4514		LB-01IN	LB-55571	–	–	2.4×10^{-2}	1.0×10^{-2}	–	8.1×10^{-4}
NLE-01VT		LB-01IN	LB-55571	–	–	2.4×10^{-2}	1.0×10^{-2}	–	8.1×10^{-4}
Cryptosporidium parvum virus 1	23								
Iwa13_5		Iwa39_6	E29_09	–	2.6×10^{-2}	1.7×10^{-2}	–	–	2.9×10^{-4}
Cucurbitaria piceae partitivirus 1	6								
Ore2		CBS176	Hrudkov	3.0×10^{-3}	3.3×10^{-5}	9.2×10^{-5}	9.3×10^{-3}	–	–

Putative recombination breakpoints were identified on CP-RdRp concatenated sequences using programs implemented in RDP4 package [44]. These analyses were done using default settings for the different programs. Putative reassortants identified by three or more methods were shown here

n Number of analysed sequences

reassortment candidates because they differ extremely in their CP identity (8 in betapartitiviruses and 9 in alphapartitiviruses) even as they are related in terms of RdRp. Six putative reassortants were identified among isolates of four different viruses, also.

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Compliance with ethical standards

Conflict of interest The author declares no conflict of interest.

Ethical approval This article does not contain any experiments with human participants or animals and is in compliance with ethical standards for research.

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