A novel deltacryptic virus identified in Allium cepa from Brazil

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Summary. – This work describes a novel partitivirus genome assembled from RNA-seq data generated from onion tissue from fields in Brazil. A new partitivirus genome composed of three dsRNAs, which was closely related to arhar cryptic virus 1, was assembled from *Allium cepa* samples from Brazil. The genomic sequences were also identified from available transcriptomic datasets of onion samples from China, Czech Republic, India, South Korea and USA. According to the species demarcation in the *Partitiviridae* family, the new virus was classified into the genus *Deltapartitivirus* with the suggested name of allium deltapartitivirus. This is the first report of the occurrence of a cryptic virus in plants of the genus *Allium*, and therefore, this work contributes to the understanding of the genetic diversity of partitiviruses that infect the genus *Allium*.

Keywords: Allium sp.; high-throughput sequencing; partitiviruses

Currently, the *Partitiviridae* family consists of 45 species distributed into five genera, and 15 species not yet classified (ICTV, 2022). Members of the genera *Alphapartitivirus* and *Betapartitivirus* infect plants or fungi, those of the genus *Gammapartitivirus* infect only fungi, of the *Deltapartitivirus* only plants, and there is only one approved species of the genus *Cryspovirus* that infects protozoa (Nibert *et al.*, 2014). Genomes consist of two or three genomic segments of double-stranded RNA packaged separately in isometric particles (30–42 nm) that are not enveloped (Pan *et al.*, 2009; Vainio *et al.*, 2018; Byrne *et al.*, 2021). The RNA-dependent RNA polymerase (RdRp) is coded on dsRNA-1, and the coat protein (CP) on dsRNA-2 (Nibert *et al.*, 2014). Occasionally partitiviruses are associated with satellite RNAs or defective RNAs (Chiba *et al.*, 2013).

Infections caused by partitiviruses in their respective hosts are persistent, and natural vectors are unknown or do not exist (Boccardo et al., 1987; Nibert et al., 2009; Vainio and Hantula, 2016; Cross et al., 2020). Fungal partitiviruses are transmitted through hyphal anastomosis and sporogenesis but can be also transmitted through contacts between hyphae (Ihrmark et al., 2002; Sasaki et al., 2006; Bhatti et al., 2011; Liu et al., 2012; Chiba et al., 2013; Xiao et al., 2014), while plant partitiviruses are transmitted through ovule and by pollen to the seed embryo (Valverde and Gutierrez, 2008; Roossinck, 2010). There are no reports of graft transmission and apparently no cell-to-cell movement and transmission occurs strictly vertically via meiosis (Boccardo et al., 1987; Ghabrial et al., 2008; Nibert et al., 2014). Members of the genus Deltapartitivirus have two or more nucleic acid segments (Sabanadzovic and Valverde, 2011) and are found at very low concentration in the host plant (Yang et al., 2022). Other additional RNAs have been found in deltapartitiviruses, which also encode for another full-length CP with an unknown function

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Abbreviations: ADp = allium deltapartitivirus; ArCV-1 = arhar cryptic virus l; CP = coat protein; ICTV = International Committee on Taxonomy of Viruses; ORF(s) = open reading frame(s); SDT = sequence demarcation tool; TSA = transcriptome shotgun assembly

(Sabanadzovic and Valverde, 2011; Kim et al., 2018; Kumar et al., 2017).

In this work, we used RNA-seq data of Allium cepa cultivated in Brazil to assemble a putatively new genomic sequence of a deltapartitivirus closely related to arhar cryptic virus 1 (ArCV-1). Three genomic sequences were assembled, molecularly characterized, and were also found in TSA datasets of onions from different countries around the world. Therefore, we first collected symptomatic tissue of onion leaves (Allium cepa L.) from producing fields in the city of Petrolândia, Pernambuco, Brazil. Total RNA was extracted using the Quick-RNA™ Plant Miniprep Kit (Zymo Research, CA, USA) according to the manufacturer's instructions and sent for High-Throughput Sequencing to Macrogen (Seoul, South Korea). Two files RB-1_1.fastq.gz (1.2 Gb), and RB-1_2.fastq. gz (1.3 Gb) were generated with a total of 5,270,386,040 reads (GC content of 43.65%, AT of 56.35%, the proportion of bases with Phred quality score higher than 20 (Q20%) was 98.72% and 30 (Q30%) was 95.85%). Also, seven transcriptome datasets (TSA prefixes GAAO, GBJZ, GBRO, GBRO, GEOY, GETF, and GHMJ) of Allium cepa were retrieved from NCBI and used to assemble complete or partial partitiviral sequences of dsRNA-1, dsRNA-2 and dsRNA-3. The bioinformatics pipeline for de novo assembly of viral genomes was performed according to Blawid et al. (2017). RdRp amino acid sequences of partitiviruses were used in multiple sequence alignments using MAFFT v.7 (Katoh and Standley, 2013). The best model of protein evolution was evaluated with ProtTest 3.4.2 (Darriba et al., 2011). Based on the AIC and BIC criteria, the LG substitution matrix with I, G and F distributions were chosen, with a confidence interval of 100%. Phylogenetic analyses were then performed with the predetermined parameters of PhyML 3.0 (Guindon et al., 2010, http:// www.atgc-montpellier.fr/phyml/execution.php). The starting tree was obtained by BioNJ and optimized by both branch length and tree topology. Branch support values (%) were estimated by the approximate likelihood ratio test (aLRT) with SH-like criteria. Phylogenetic tree was visualized and edited with iTOL v4 (https://itol. embl.de/tree/) (Letunic and Bork, 2021). The Sequence Demarcation Tool (SDTv.1.2) (Muhire et al., 2014) was used to analyze multiple alignments at the amino acid level of the RdRp (dsRNA-1, 502 aa) and the CPs (dsRNA-2, 345 aa; dsRNA-3, 338 aa) of partitiviruses. GenBank Acc. Nos. of the partitivirus sequences used for analysis in this publication are in Supplementary Table S1.

As a result, three segments (dsRNA1- OP313027, dsRNA2- OP313028, dsRNA3- OP313029) were assembled from onion samples from Brazil, which were closely related to deltapartitiviruses. According to ICTV, the binomial name allium deltapartitivirus (ADp) was suggested. dsRNA-1 consists of 1734 bp and has an ORF of 1509 bp (nucleotide position 103 to 1611) that putatively encodes a 502 aa RdRp (MW 59.41 kDa). Blast analyses revealed the highest amino acid sequence identity of 77.80% (94% coverage) with the unclassified RdRp sequence of ArCV-1 (NC_024014) followed by 74% with the RdRp aa sequence of rosa multiflora cryptic virus (RMCV, ABV89762). SDT analyses using the complete ADp RdRp amino acid sequence revealed the highest percentage identities of 42.8-77.3 with unclassified RdRp deltapartitivirus sequences (ArCV-1, NC_024014; fragaria chiloensis cryptic virus, FClCV, NC_009519; RMCV; rose cryptic virus 1, RoCV1, NC_010346 and others) followed by 37 to 39.3% identities with deltapartitivirus sequences (beet cryptic virus 2, NC_038846; fig cryptic virus, NC_015494; pepper cryptic virus 1, NC_037095; pepper cryptic virus 2, NC_034159) (Supplementary Fig. S1). Currently the Partitiviridae family is divided into five genera corresponding to the clustering of RdRp sequences (Nibert et al., 2009; Nibert et al., 2014). Based on partitiviral RdRp-like sequences phylogenetic analysis, unclassified deltapartitiviruses and sorted deltapartitiviruses form a monophyletic group (Liu et al. 2012; Nibert et al., 2014; Kumar et al., 2017). Indeed, our RdRp phylogenetic analysis using RdRp amino acid sequences of sorted and unclassified partitiviruses also placed ADp closely related to unclassified deltapartitiviruses (Fig. 1).

Furthermore, the 5' ends of ADp dsRNAs nucleotide sequences have the conserved sequence 5'-GAUAAU GAUC-3', also found in some unclassified deltapartitiviruses. This consensus follows the described consensus 5'-GAWWWUNMYC-3' (Nibert *et al.*, 2014) found for all deltapartitiviruses so far. Finally, several structural RdRp (A-G) motifs (Bruenn, 1993; Xie *et al.*, 1993; Bartholomäus *et al.*, 2016; Jia and Gong, 2019) were found: Motifs A (DX₄D), B (P/A/SGX₃TX₄SX₂N), the C box (GDD), D (KS, KC, KT or KL), E (LX₂₂P/L/TE/K/R), F (PX₆R X₂IX₇KXR) and G (AG, AE, VE. AD or TG) (Supplementary Fig. S2).

ADp dsRNA-2 consists of 1474 bp and has a 1038 bp ORF-2 (345 aa, MW ~38.29 kDa) that putatively codes for a coat protein (CP1). Blastp analysis of the CP1 amino acid sequence showed the highest amino acid sequence identity of 45.99% with the ArCV-1 coat protein (NC_024011, cover 97%). dsRNA-3 is 1413 bp long and has an ORF consisting of 1017 bp that also putatively codes for another CP (CP2, 338 aa, MW ~37.9 kDa). The highest amino acid identity of 47.9% (97% coverage, Blastp) was found with the hypothetical protein LR48_Vigan07g013900 from Vigna angulares (KOM46435) followed by 50.2% (86% coverage) with the CP2 of ArCV-1 (NC_024010). SDT matches using CP1 and CP2 sequences at the amino acid level revealed higher identities to unclassified partitiviruses (Supplementary Fig. S3), in which the CP1 aa sequence shared 45.7% identity with the CP1 sequence of ArCV-1 (NC_024011), and

43.8% with the FClCV sequence (NC_009520). The CP2 of ADp shares 47.6% identities with the CP2 sequence of ArCV-1 (NC_024010), and 45.2% with the CP2 of RoCV1 (NC_010348) and RMCV (EU024676). When CP1 and CP2 aa sequences were compared with other partitivirus sequences, the highest identity between 19.0% to 43.3% were observed (Supplementary Fig. S3). The CP1/CP2 length of dsRNA-2 and dsRNA-3 are in the range of other deltapartitiviruses, which varies from 1415–1598 bp (337–430 aa). We also performed a phylogenetic analysis using CP1 and CP2 amino acid sequences of partitiviruses (Supplementary Fig. S4) besides identifying the low identities among partitiviruses. ADp CPs grouped with unclassified partitiviruses sequences (ArCV-1, FClCV, RoCV1, RMCV), show-

ing a close phylogenetic relationship with unclassified partitivirids. The CPs sequences of ADp grouped into two clades formed with CP1 and CP2 sequences, respectively. The closest relationship of ADp CP1/CP2 was found with the CP1/CP2 of ArCV-1.

Concerning the poly (A) uninterrupted stretches at their 3'-terminal ends, deltapartitiviruses are known to have 12–19 A residues in the last 50 nt of the 3'-terminal of dsRNA-1 and 9–18 of dsRNA-2 sequences (Nibert *et al.,* 2014; An *et al.,* 2017). ADp dsRNA-1, dsRNA-2 and dsRNA-3 have 11, 14 and 11 A residues at its last 50 nt, respectively.

Interestingly, we also were able to assemble RdRp, CP1 and CP2 sequences of ADp from TSA datasets of onions from China, Czech Republic, India, South Korea and USA,



Fig. 1

Phylogenetic relationships between members of the *Partitiviridae* family and unclassified partitiviruses based on the amino acid sequences of the RdRp region

The newly assembled sequence of Allium deltapartitivirus is shown in bold. Nodes display branch support values (%).

which shared aa identities of 84-100% with the corresponding sequences (Supplementary Table S2). Pairwise multiple sequence comparisons at the amino acid level showed identities as high as 99.7% and 99.4% in the CP1 and CP2 regions, respectively, with other sequences derived from transcriptomic data. This suggests that ADp is widespread in *A. cepa* indicating that ADp might have co-evolved with the host over a long time.

So far, unclassified partitiviruses have been detected in asymptomatic and symptomatic plants, as found for RoCV1 (James *et al.*, 2015), and some authors have suggested that partitivirus possibly plays a role in diseaseassociated virus complex (Martin and Tzanetakis, 2008). Although we found ADp from symptomatic onion leaves, future studies should be performed to clarify whether partitivirus infection plays a role in disease expression in onions. This work contributes to the understanding of the diversity of partitiviruses in onions and enunciates the first genome of a partitivirus infecting plants of *Allium cepa*.

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Supplementary information is available in the online version of the paper.

References

- Bartholomäus A, Wibberg D, Winkler A, Pühler A, Schlüter A, Varrelmann M (2016): Deep sequencing analysis reveals the mycoviral diversity of the virome of an avirulent isolate of Rhizoctonia solani AG-2-2 IV. PLoS One, 11, e0165965. <u>https://doi.org/10.1371/journal. pone.0165965</u>
- Bhatti MF, Jamal A, Petrou MA et al. (2011): The effects of dsRNA mycoviruses on growth and murine virulence of Aspergillus fumigatus. Fungal Genet. Biol. 48, 1071-1075. https://doi.org/10.1016/j.fgb.2011.07.008
- Blawid R, Silva JMF, Nagata T (2017): Discovering and sequencing new plant viral genomes by next-generation sequencing: description of a practical pipeline. Ann. Appl. Biol. 170, 301–314. <u>https://doi.org/10.1111/aab.12345</u>
- Boccardo G, Lisa V, Luisoni E, Milne RG (1987): Cryptic Plant Viruses. In Maramorosch K, Murphy FA, Shatkin AJ (Eds.): Advances in Virus Research. Academic Press, pp. 171–214. <u>https://doi.org/10.1016/S0065-3527(08)60477-7</u>
- Byrne M, Kashyap A, Esquirol L et al. (2021): The structure of a plant-specific partitivirus capsid reveals a unique coat protein domain architecture with an intrinsically

disordered protrusion. Commun. Biol. 4, 1–8. <u>https://doi.org/10.1038/s42003-021-02687-w</u>

- Bruenn JA (1993): A closely related group of RNA-dependent RNA polymerases from double-stranded RNA viruses. Nucleic Acids Res. 21, 5667–5669. <u>https://doi. org/10.1093/nar/21.24.5667</u>
- Chiba S, Lin Y-H, Kondo H et al. (2013): Effects of defective interfering RNA on symptom induction by, and replication of, a novel partitivirus from a phytopathogenic fungus, Rosellinia necatrix. J. Virol. 87, 2330–2341. <u>https://doi. org/10.1128/JVI.02835-12</u>
- Cross ST, Maertens BL, Dunham TJ et al. (2020): Partitiviruses infecting Drosophila melanogaster and Aedes aegypti exhibit efficient biparental vertical transmission. J. Virol. 94, e01070-20. <u>https://doi.org/10.1128/JVI.01070-20</u>
- Darriba D, Taboada GL, Doallo R, Posada D (2011): ProtTest 3: fast selection of best-fit models of protein evolution. Bioinformatics 27, 1164–1165. <u>https://doi.org/10.1093/</u> bioinformatics/btr088
- Ghabrial SA, Ochoa WF, Baker TS, Nibert ML (2008): Partitiviruses: General Features. In Encyclopedia of Virology. Elsevier, pp. 68–75. <u>https://doi.org/10.1016/B978-012374410-4.00573-2</u>
- Guindon S, Dufayard J-F, Lefort V et al. (2010): New Algorithms and methods to estimate Maximum-Likelihood phylogenies: Assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321. <u>https://doi.org/10.1093/sysbio/ syq010</u>
- ICTV (2022): International Committee on Taxonomy of Viruses, https://talk.ictvonline.org/taxonomy/. Accessed on 22 June, 2022.
- Ihrmark K, Johannesson H, Stenström E, Stenlid J (2002): Transmission of double-stranded RNA in Heterobasidion annosum. Fungal Genet. Biol. 36, 147–154. <u>https://doi.org/10.1016/S1087-1845(02)00011-7</u>
- James D, Phelan J, Varga A et al. (2015): First report of rose cryptic virus 1 in rosa plants in Canada. Plant Dis. 99,558–558. <u>https://doi.org/10.1094/PDIS-09-14-0921-PDN</u>
- Jia H, Gong P (2019): A structure-function diversity survey of the RNA-dependent RNA polymerases from the positivestrand RNA viruses. Front. Microbiol. 10, 1945. <u>https:// doi.org/10.3389/fmicb.2019.01945</u>
- Katoh K, Standley DM (2013): MAFFT Multiple Sequence Alignment Software Version 7: Improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010
- Kim H, Park D, Hahn Y (2018): Identification of novel RNA viruses in alfalfa (Medicago sativa): an Alphapartitivirus, a Deltapartitivirus, and a Marafivirus. Gene 638, 7-12. https://doi.org/10.1016/j.gene.2017.09.069
- Kumar S, Subbarao BL, Kumari R, Hallan V (2017): Molecular characterization of a novel cryptic virus infecting pigeonpea plants. PLoS One 12(8), e0181829. <u>https:// doi.org/10.1371/journal.pone.0181829</u>
- Letunic I, Bork P (2021): Interactive Tree Of Life (iTOL): v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 49, W293-W296. <u>https://doi. org/10.1093/nar/gkab301</u>

- Liu H, Fu Y, Xie J et al. (2012): Discovery of novel dsRNA viral sequences by in silico cloning and implications for viral diversity, host range and evolution. PLoS One 7, e42147. https://doi.org/10.1371/journal.pone.0042147
- Martin RR, Tzanetakis IE (2008): First report of Rosa multiflora cryptic virus in Rosa multiflora in the Eastern United States. Plant Dis. 92, 1706–1706. <u>https://doi.org/10.1094/</u> <u>PDIS-92-12-1706B</u>
- Muhire BM, Varsani A, Martin DP (2014): SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9, e108277. <u>https://doi.org/10.1371/journal.pone.0108277</u>
- Nibert ML, Ghabrial SA, Maiss E et al. (2014): Taxonomic reorganization of family Partitiviridae and other recent progress in partitivirus research. Virus Res. 188, 128–141. https://doi.org/10.1016/j.virusres.2014.04.007
- Nibert ML, Woods KM, Upton SJ, Ghabrial SA (2009): Cryspovirus: a new genus of protozoan viruses in the family Partitiviridae. Arch. Virol. 154, 1959–1965. <u>https://doi.org/10.1007/s00705-009-0513-7</u>
- Pan J, Dong L, Lin L et al. (2009): Atomic structure reveals the unique capsid organization of a dsRNA virus. Proc. Natl. Acad. Sci. U.S.A 106, 4225–4230. <u>https://doi. org/10.1073/pnas.0812071106</u>
- Roossinck MJ (2010): Lifestyles of plant viruses. Philosophical transactions of the Royal Society B: Biol. Sci. 365, 1899–1905. <u>https://doi.org/10.1098/rstb.2010.0057</u>

- Sabanadzovic S, Valverde RA (2011): Properties and detection of two cryptoviruses from pepper (Capsicum annuum). Virus Genes 43, 307-312. <u>https://doi.org/10.1007/s11262-011-0634-4</u>
- Sasaki A, Kanematsu S, Onoue M et al. (2006): Infection of Rosellinia necatrix with purified viral particles of a member of Partitiviridae (RnPV1-W8). Arch. Virol. 151,697-707. <u>https://doi.org/10.1007/s00705-005-0662-2</u>
- Vainio EJ, Chiba S, Ghabrial SA et al (2018): ICTV Virus Taxonomy Profile: Partitiviridae. J. Gen. Virol. 99, 17-18. <u>https://doi.org/10.1099/jgv.0.000985</u>
- Vainio EJ, Hantula J (2016): Taxonomy, biogeography and importance of Heterobasidion viruses. Virus Res. 219, 2-10. https://doi.org/10.1016/j.virusres.2015.10.014
- Valverde RA, Gutierrez DL (2008): Molecular and biological properties of a putative partitivirus from jalapeño pepper (Capsicum annuum L.). Rev. Mex.i Fitopatol. 26, 1–6.
- Xiao X, Cheng J, Tang J et al. (2014): A novel partitivirus that confers hypovirulence on plant pathogenic fungi. J. Virol. 88, 10120-10133. <u>https://doi.org/10.1128/JVI.01036-14</u>
- Xie W, Antoniw J, White R (1993): Nucleotide sequence of beet cryptic virus 3 dsRNA2 which encodes a putative RNAdependent RNA polymerase. J. Gen. Virol. 74, 1467–1470. https://doi.org/10.1099/0022-1317-74-7-1467
- Yang J, Peng Y, Kong W (2022): Identification and complete genome sequence of Mulberry cryptic. Arch. Virol. 167, 687-690. <u>https://doi.org/10.1007/s00705-021-05350-1</u>

SUPPLEMENTARY INFORMATION

A novel deltacryptic virus identified in Allium cepa from Brazil

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| Genera | Species (RdRp) | GenBank Acc. No. | Viruses acronyms |
|---------------------------|--|------------------|------------------|
| | Amasya cherry disease-associated mycovirus | NC_006441 | AcPV |
| | Beet cryptic virus 1 | NC_011556 | BCV-1 |
| | Carrot cryptic virus | NC_038824 | CaCV |
| | Chondrostereum purpureum cryptic virus 1 | NC_038916 | CpCV1 |
| | Flammulina velutipes browning virus | NC_038826 | FvBV |
| A la la an a stitiation a | Heterobasidion partitivirus 1 | NC_038827 | HetPV1 |
| Alphapartitivirus | Heterobasidion partitivirus 3 | NC_038836 | HetPV3 |
| | Heterobasidion partitivirus 12 | NC_038829 | HetPV12 |
| | Heterobasidion partitivirus 13 | NC_038831 | HetPV13 |
| | Heterobasidion partitivirus 15 | NC_038834 | HetPV15 |
| | Vicia cryptic virus | NC_007241 | VCV |
| | White clover cryptic virus 1 | NC_006275 | WCCV1 |

Supplementary Table S1. GenBank Acc. No. of partitivirus sequences used for analysis in this publication

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Supplementary Table S1 (continued)

| Genera | Species (RdRp) | GenBank Acc. No. | Viruses acronyms |
|-------------------|---|------------------|------------------|
| | Atkinsonella hypoxylon virus | NC_003470 | AhV |
| | Cannabis cryptic virus | NC_031134 | CCV |
| | Ceratocystis resinifera virus 1 | NC_010755 | CrV1 |
| | Crimson clover cryptic virus 2 | NC_038837 | CCCV2 |
| | Dill cryptic virus 2 | NC_021147 | DCV-2 |
| | Fusarium poae virus 1-240374 | NC_030882 | FpV1-240374 |
| . | Heterobasidion partitivirus 2 | NC_038839 | HEPV2 |
| Betapartitivirus | Heterobasidion partitivirus 7 | NC_035485 | HEPV7 |
| | Heterobasidion partitivirus 8 | NC_038841 | HetPV8 |
| | Hop trefoil cryptic virus 2 | NC_021098 | HTCV2 |
| | Pleurotus ostreatus virus 1 | NC_006961 | PoV1 |
| | Primula malacoides virus China/Mar2007 | NC_013109 | PmV1 |
| | Red clover cryptic virus 2 | NC_021096 | RCCV2 |
| | White clover cryptic virus 2 | NC_021094 | WCCV2 |
| Cryspovirus | Cryptosporidium parvum virus 1 | NC_038843 | CSpV1 |
| | Beet cryptic virus 2 | NC_038846 | BCV2 |
| | Fig cryptic virus | NC_015494 | FCV |
| Deltapartitivirus | Pepper cryptic virus 1 | NC_037095 | PCV1 |
| | Pepper cryptic virus 2 | NC_034159 | PCV2 |
| | Aspergillus ochraceous virus | NC 043396 | AoV |
| | Discula destructiva virus 1 | NC 002797 | DdV1 |
| | Discula destructiva virus 2 | NC 003710 | DdV2 |
| | Fusarium solani virus 1 | NC_003885 | FsV1 |
| Gammapartitivirus | Gremmeniella abietina RNA virus MS1 | NC_004018 | GaRV-MS1 |
| | Ophiostoma partitivirus 1 | NC_038918 | OPV1 |
| | Penicillium stoloniferum virus S | NC 005976 | PsV-S |
| | Penicillium stoloniferum virus F | NC_007221 | PsV-F |
| | Allium deltapartitivirus | OP313027 | ADp |
| | Arceuthobium sichuanense virus 5 | BK059272 | - |
| | Arceuthobium sichuanense virus 6 | BK059275 | - |
| | Arhar cryptic virus-1 | NC_024014 | ArCV-1 |
| | Camellia cryptic virus 1 | MH898482 | _ |
| | Conifer deltapartitivirus | OW524923 | - |
| | Fragaria chiloensis cryptic virus | NC_009519 | FClCV |
| | Freshwater macrophyte associated partiti-like virus 3 | ON125117 | FMAPLV3 |
| | Interrupted club-moss deltapartitivirus | OW528592 | - |
| Unclassified | Japanese plum-vew deltapartitivirus | OW528994 | - |
| deltapartitivirus | Pistacia cryptic virus | MT334613 | _ |
| | Port Orford cedar deltapartitivirus | OW528599 | _ |
| | Racomitrium varium deltapartitivirus RNA1 | OW528603 | RVDN1 |
| | Raphanus sativus cryptic virus 2 | NC_010343 | RsCV-2 |
| | Rosa multiflora cryptic virus | EU024675 | RMCV |
| | Rose crvptic virus 1 | NC 010346 | RoCV1 |
| | Silvergrass cryptic virus 1 | MZ751043 | SgCV-1 |
| | Subalpine fir deltapartitivirus | OW528601 | - |
| | Yunnan larch deltanartitivirus | OW528604 | |
| | 1 unitan iur en uenapar intern us | 011020004 | |

| | Supplementary Table S1 (continued) | | |
|-------------------|--|------------------|------------------|
| Genera | Species (CP-1/CP-2) | GenBank Acc. No. | Viruses acronyms |
| | Amasya cherry disease-associated mycovirus | NC_006440, CP-1 | AcPV |
| Alphapartitivirus | Beet cryptic virus 1 | NC_011557, CP-1 | BCV1 |
| | Carrot cryptic virus | NC_038823, CP-1 | CaCV |
| | Chondrostereum purpureum cryptic virus 1 | NC_038915, CP-1 | CpCV1 |
| | Flammulina velutipes browning virus | NC_038825, CP-1 | FvBV |
| | Heterobasidion partitivirus 1 | NC_038828, CP-1 | HetPV1 |
| | Heterobasidion partitivirus 3 | NC_038835, CP-1 | HetPV3 |
| | Heterobasidion partitivirus 12 | NC_038830, CP-1 | HetPV12 |
| | Heterobasidion partitivirus 13 | NC_038832, CP-1 | HetPV13 |
| | Heterobasidion partitivirus 15 | NC_038833, CP-1 | HetPV15 |
| | Vicia cryptic virus | NC_007242, CP-1 | VCV |
| | White clover cryptic virus 1 | NC_006276, CP-1 | WCCV1 |
| | Atkinsonella hypoxylon virus | NC_003471, CP-1 | AhV |
| | Cannabis cryptic virus | NC_031130, CP-1 | CCV |
| | Ceratocystis resinifera virus 1 | NC_010754, CP-1 | CrV1 |
| | Crimson clover cryptic virus 2 | NC_038838, CP-1 | CCCV2 |
| | Dill cryptic virus 2 | NC_021148, CP-1 | DCV2 |
| | Fusarium poae virus 1-240374 | NC_030877, CP-1 | FpV1-240374 |
| | Heterobasidion partitivirus 2 | NC_038840, CP-1 | HEPV2 |
| Betapartitivirus | Heterobasidion partitivirus 7 | NC_035486, CP-1 | HEPV7 |
| | Heterobasidion partitivirus 8 | NC_038842, CP-1 | HetPV8 |
| | Hop trefoil cryptic virus 2 | NC_021099, CP-1 | HTCV2 |
| | Pleurotus ostreatus virus 1 | NC_006960, CP-1 | PoV1 |
| | Primula malacoides virus China/Mar2007 | NC_013110, CP-1 | PmV1 |
| | Red clover cryptic virus 2 | NC_021097, CP-1 | RCCV2 |
| | White clover cryptic virus 2 | NC_021095, CP-1 | WCCV2 |
| Cryspovirus | Cryptosporidium parvum virus 1 | NC_038844, CP-1 | CSpV1 |
| | Beet cryptic virus 2 | NC_038845, CP-1 | BCV2 |
| | Beet cryptic virus 2 | NC_038847, CP-2 | BCV2 |
| Deltapartitivirus | Fig cryptic virus | NC_015495, CP-1 | FCV |
| Pepper Pepper | Pepper cryptic virus 1 | NC_037096, CP-1 | PCV1 |
| | Pepper cryptic virus 2 | NC_034167, CP-1 | PCV2 |
| | Aspergillus ochraceous virus | NC_043397, CP-1 | AoV |
| Gammapartitivirus | Discula destructiva virus 1 | NC_002800, CP-1 | DdV1 |
| | Discula destructiva virus 2 | NC_003711, CP-1 | DdV2 |
| | Fusarium solani virus 1 | NC_003886, CP-1 | FsV1 |
| | Gremmeniella abietina RNA virus MS1 | NC_004019, CP-1 | GaRV-MS1 |
| | Ophiostoma partitivirus 1 | NC_038917, CP-1 | OPV1 |
| | Penicillium stoloniferum virus F | NC_007222, CP-1 | PsV-S |
| | Penicillium stoloniferum virus S | NC 005977 CP-1 | PsV-F |

Supplementary Table SI (continued)

| Supplementary Table S1 (continue |
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| Genera | Species (CP-1/CP-2) | GenBank Acc. No. | Viruses acronyms |
|-----------------------------------|---|------------------|------------------|
| | Allium deltapartitivirus | OP313028, CP-1 | ADp |
| | Allium deltapartitivirus | OP313029, CP-2 | ADp |
| | Arceuthobium sichuanense virus 5 | BK059273, CP-1 | - |
| | Arceuthobium sichuanense virus 5 | BK059274, CP-2 | - |
| | Arceuthobium sichuanense virus 6 | BK059276, CP-1 | - |
| | Arceuthobium sichuanense virus 6 | BK059277, CP-2 | - |
| | Arhar cryptic virus-1 | NC_024011, CP-1 | ArCV-1 |
| | Arhar cryptic virus-1 | NC_024010, CP-2 | ArCV-1 |
| | Camellia cryptic virus 1 | MH898483, CP-1 | - |
| | Camellia cryptic virus 1 | MH898484, CP-2 | - |
| | Conifer deltapartitivirus | OW524924, CP-2 | |
| | Fragaria chiloensis cryptic virus | NC_009521, CP-1 | FClCV |
| | Fragaria chiloensis cryptic virus | NC_009520, CP-2 | FClCV |
| | Interrupted club-moss deltapartitivirus | OW528636, CP-1 | _ |
| | Japanese plum-yew deltapartitivirus | OW528640, CP-1 | _ |
| Unclassified deltapartitivirus | Japanese plum-yew deltapartitivirus | OW527828, CP-2 | - |
| | Pistacia cryptic virus | MT334607, CP-1 | - |
| | Pistacia cryptic virus | MT334606, CP-2 | - |
| | Port Orford cedar deltapartitivirus | OW525634, CP-1 | - |
| | Port Orford cedar deltapartitivirus | OW528600, CP-2 | - |
| | Raphanus sativus cryptic virus 2 | NC_010344, CP-1 | RsCV-2 |
| | Raphanus sativus cryptic virus 2 | NC_010345, CP-2 | RsCV-2 |
| | Rosa multiflora cryptic virus | EU024677, CP-1 | RMCV |
| | Rosa multiflora cryptic virus | EU024676, CP-2 | RMCV |
| | Rose cryptic virus 1 | NC_010347, CP-1 | RoCV1 |
| | Rose cryptic virus 1 | NC_010348, CP-2 | RoCV1 |
| | Silvergrass cryptic virus 1 | MZ751045, CP-1 | SgCV-1 |
| | Silvergrass cryptic virus 1 | MZ751044, CP-2 | SgCV-1 |
| | Subalpine fir deltapartitivirus | OW528627, CP-1 | - |
| | Subalpine fir deltapartitivirus | OW528628, CP-2 | - |
| | Yunnan larch deltapartitivirus | OW528634, CP-1 | - |
| | Yunnan larch deltapartitivirus | OW525367, CP-2 | - |

| TSA/Bioproject Acc. No. | Country | RdRp, CP1, CP2 (aa-length) | % Identities at aa level |
|---|----------------|-------------------------------|--------------------------|
| GAAO01015175 GAAO01016116 GAAO01015731/ <u>PRJNA175449</u> | USA | 475 345 338 | 84.0 99.4 100 |
| GBJZ01114532 GBJZ01156044 GBJZ01160704/ PRJNA248253 | India | 502 345 338 | 95.8 99.1 99.7 |
| - GEOY01111130 GEOY01067339/ <u>PRJNA310814</u> | China | - 345 338 | 99.4 99.1 |
| GETF01033505 GETF01028131 GETF01027781/ PRJNA298934 | South Korea | 475 345 338 | 84.0 99.4 99.4 |
| GHMJ01083671 GHMJ01021247 GHMJ01079223 PRJNA542932 | Czech Republic | 502 345 338 | 95.8 98.8 100 |

Supplementary Table S2. List of TSA datasets used to assemble RdRp, CP1 and CP2 ORFs from Allium cepa samples and results of pairwise alignments using MAFFT



Supplementary Fig. S1

SDT analysis of RdRp regions of partitiviruses



Supplementary Fig. S2

Conserved RdRps motifs found by multiple sequence alignments using partitivirus sequences



Supplementary Fig. S3

SDT analysis of CP regions of partitiviruses



Supplementary Fig. S4

Phylogenetic relationships between members of the *Partitiviridae* family and unclassified partitiviruses based on the amino acid sequences of the CP regions

The newly assembled sequence of Allium deltapartitivirus is shown in bold. Nodes display branch support values (%).