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**Part 1:** **TITLE, AUTHORS, APPROVALS, etc**

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| **Code assigned:** | **2020.002F** |  |
| **Short title:** Create one new family *Curvulaviridae*, one new genus *Orthocurvulavirus* and eight new species | | |
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**Author(s) and email address(es)**

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**List the ICTV Study Group(s) that have seen this proposal**

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| *Partitiviridae* Study Group |

**ICTV study group comments and response of proposer**

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| The proposal has been written by EJV and finalized according to comments by *Partitiviridae* SG members. |

**Authority to use the name of a living person**

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| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
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**Submission dates**

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| Date first submitted to SC Chair |  |
| Date of this revision (if different to above) |  |

**ICTV-EC comments and response of the proposer**

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**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

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| 2020.002F.R.Curvulaviridae.xlsx |

**Abstract**

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| After the discovery of Curvularia thermal tolerance virus (CThTV) in 2007 (Márquez et al., 2007), related dsRNA viruses with bisegmented genomes have been identified in many ascomycetous and basidiomycetous fungi. Several published reports have described their genome organization, evolutionary relationships and biological characteristics, but these viruses remain unclassified by the ICTV. Based on phylogenetic analysis, these viruses are evolutionarily related to but distinct from members of classified virus families, which warrants their official recognition as a separate family. We propose the creation of a new family *Curvulaviridae* encompassing eight species in the new genus *Orthocurvulavirus.* |

**Text of proposal**

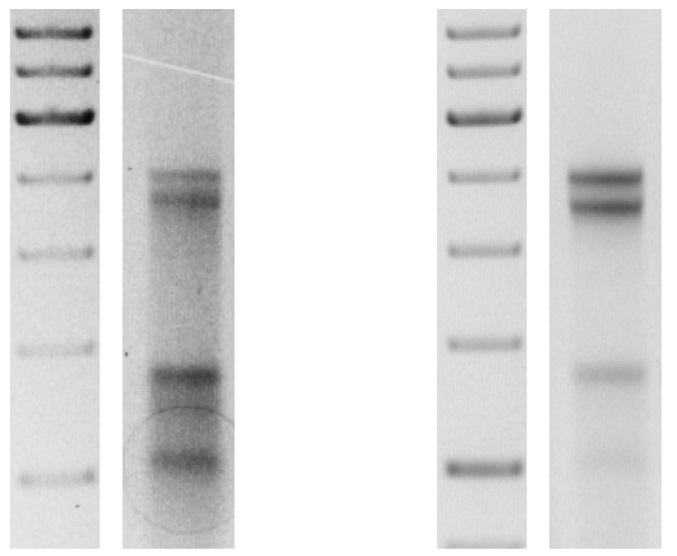
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Table 2 (see Supporting evidence) lists additional virus isolates with incomplete or unpublished genome sequences (Botella et al. 2015; Bartholomäus et al., 2016; Nerva et al., 2016, 2019; Ong et al., 2018; Zhu et al., 2018; Picarelli et al., 2019), considered as members of probable species in the newly proposed family.  **Table 1.** Proposed new species   |  |  |  | | --- | --- | --- | | **New species** | **Exemplar isolate** | **GenBank sequence accession number(s) of reference isolate:** | | *Curvularia orthocurvulavirus 1* | Curvularia thermal tolerance virus | EF120984, EF120985 | | *Fusarium graminearum orthocurvulavirus* | Fusarium graminearum dsRNA mycovirus 4 | GQ140627, GQ140628 | | *Heterobasidion orthocurvulavirus* | Heterobasidion RNA virus 6 | HQ189459, MK468678 | | *Lactarius rufus orthocurvulavirus 1* | Lactarius rufus RNA virus 1 | MT130415, MT130416 | | *Lactarius tabidus orthocurvulavirus 1* | Lactarius tabidus RNA virus 1 | KT733084, MG596785 | | *Rhizoctonia solani orthocurvulavirus 1* | Rhizoctonia solani dsRNA virus 1 | JX976612, JX976613 | | *Sclerotium hydrophilum orthocurvulavirus 1* | Sclerotium hydrophilum virus 1 | KU886558, KU886559 | | *Trichoderma harzianum orthocurvulavirus 1* | Trichoderma harzianum bipartite mycovirus 1 | MH536648, MH536649 |   **Genome:**  The genome comprises two dsRNA segments of ~2.0-2.4 kbp (dsRNA1) and ~1.7-2.0 kbp (dsRNA2), which are detectable in infected tissue (Fig. 1). The 5′-termini of both dsRNAs are typically conserved, and the viruses do not have poly(A) tracts at the plus-strand 3′-terminus of their genome segments. The GC content of dsRNA1 ranges from 49.0% to 56.8%, and the GC content of dsRNA2 from 54.5% to 62.5%. The larger genome segment encodes a putative RdRp with characteristic conserved motifs including the GDD core domain (e.g., Yu et al., 2009; Vainio et al., 2012), and the smaller genome segment encodes 1–2 proteins with unknown function (Fig. 2).  **Phylogenetic relationships:**  Phylogenetic analysis of RdRp sequences (Fig. 3) shows that the proposed family members form a highly supported cluster distinct from other dsRNA viruses and distantly related to members of families *Amalgaviridae* (Sabanadzovic et al., 2009) and *Partitiviridae* (Vainio et al., 2008), and a group of unclassified viruses tentatively named as “unirnaviruses” (Kotta-Loizou et al., 2015). Figure 4 presents identity scores based on global pairwise alignments of the RdRp and a protein encoded by ORF1 of dsRNA2. The polymerase sequence identities among group members are ≥25%. Viruses from different host genera share up to 82% RdRp aa sequence identities, as exemplified by Penicillium aurantiogriseum bipartite virus 1 and Myriodontium keratinophilum bipartite virus 1 (Nerva et al., 2016, 2019; Fig. 4). Polymerase identity scores between proposed group members and viruses classified in other taxa are clearly lower: ~13-18% for “unirnaviruses, ~10-13% for *Amalgaviridae* and ~7-15% for *Partitiviridae* members (data not shown).  Within-species variation has been examined in several proposed or probable members of the new family*.* Partial RdRp aa sequences of HetRV6 from four different host species of *Heterobasidion* in Eurasia and North America shared ≥89% RdRp aa sequence identities (Vainio et al., 2012), and Lactarius rufus RNA virus 1 (LrRV1) isolates from two different forest sites in Southern Finland shared ≥94% RdRp aa sequence identities (Sutela and Vainio 2020), whereas partial RdRp aa sequences of Gremmeniella abietina RNA virus 6 (GaRV6) from the Spanish *Gremmeniella abietina* population were all identical (Botella et al., 2015). Partial unpublished polymerase sequences of eight isolates of CThTV are available in GenBank (accessions KT012670–77). These sequences share 94–99% BlastN sequence identity with the published original CThTV sequence (Márquez et al., 2007), which has a unique dsRNA1 organization encoding an RdRp employing two overlapping reading frames (Fig. 2). However, the partial CThTV sequences of other sequenced isolates lack an adenine nucleotide at position 1227, which restores the reading frame and results in a continuous long ORF resembling that of other members of the proposed family.  **Virus particles:**  Spherical virions of 26–29 nm have been identified with CThTV infection (Márquez et al., 2007). No electron micrographs are available for other isolates of the proposed family, except from hosts with mixed virus infection (Yu et al., 2009; Nerva et al., 2016).  **Biological and epidemiological properties:**  CThTV is reported uniquely involved in a three-way symbiosis among the virus, the grass *Dichanthelium lanuginosum* and the endophyte fungus *Curvularia protuberata* (Márquez et al., 2007), allowing the host plant to grow under harsh conditions of geothermal soil in the Yellowstone National park. None of the other group members known to date have been associated with thermal tolerance. Zheng et al. (2013) report that the host isolate of Rhizoctonia solani dsRNA virus 1 had a slower growth rate and reduced pathogenicity compared to virulent host strains.  CThTV is vertically transmitted in the conidiospores of *C. protuberata* (Márquez et al., 2007), and HetRV6 is transmitted via host basidiospores (Vainio et al., 2015). HetRV6 is also laterally transmitted between different species of the *Heterobasidion annosum* complex during hyphal contact in the laboratory (Vainio et al., 2012). In the case of HetRV6, infections by conspecific virus isolates are not stable in the same fungal host (Vainio et al., 2015). However, a mixed infection by two CThTV-like virus isolates sharing ~55% RdRp aa sequence identity has been observed in a single strain of *Sclerotium rolfsii* (Zhu et al., 2018; Table 1).  **NEW GENUS: *Orthocurvulavirus***  Based on phylogenetic analysis, members of the proposed family *Curvulaviridae* form a highly supported clade distinct from classified virus taxa. We suggest including all currently known members in a single genus named as *Orthocurvulavirus*.As the coding strategy in dsRNA1 of the first characterized virus representing the group, CThTV, is untypical of the new genus, *Fusarium graminearum orthocurvulavirus* is proposed as the type species. The total number of species (including the type species) that the genus will contain is eight.  **Origin of the new genus name:**  *Curvula*: from *Curvularia protuberata*, host of the first characterized virus representing the group  **Reasons to justify the choice of the type species:**  The second reported virus of the genus and family with a characteristic dsRNA1 coding strategy (one ORF).  **Species demarcation criteria in the new genus:**  As genus *Orthocurvulavirus* is newly proposed, there are no pre-existing criteria for species demarcations within it. These criteria are newly established here and in the Supporting evidence. Viruses from different host genera share up to 82% identities for the RdRp sequences, whereas conspecific virus isolates show higher than 89% RdRp aa sequence identities. Based on this, the suggested species delimitation threshold is set to 85%.  The proposed species demarcation criteria for the genus *Orthocurvulavirus* are:   1. ≤ 85% aa-sequence identity in the RdRp 2. Differences in natural host range   **NEW FAMILY: *Curvulaviridae***  It has been repeatedly suggested in scientific literature that CThTV and related viruses represent a new family-level taxon distinct from extant virus families (e.g., Vainio et al., 2012; Nibert et al., 2014; Picarelli et al., 2019). This view is supported by multiple molecular and biological criteria as described above and in the Supporting evidence. We propose to create a new family with one new genus within the order *Durnavirales.*  Proposed names for the new family and genus are *Curvulaviridae* and *Orthocurvulavirus,* respectively.  Similarities with the extant taxa:  These viruses share bisegmented dsRNA genome with fungal viruses in the families *Partitiviridae* and *Megabirnaviridae*, and genus *Botybirnavirus.* However, megabirnaviruses and botybirnaviruses have a substantially larger genome size and different genome organization (Wu et al., 2012; Sato et al., 2019), and megabirnaviruses are currently classified in order *Ghabrivirales*. Within *Durnavirales*, partitiviruses have a similar genome size and organization as members of the proposed family, but phylogenetic analysis places them in an evolutionarily distinct clade (Fig. 3). Members of family *Picobirnaviridae* are evolutionarily more distant from viruses in the proposed family and are not known to infect fungi (Delmas et al., 2019). Based on sequence identity scores and phylogenetic analysis of viral RdRp, viruses of the proposed family *Curvulaviridae* are evolutionarily closest to “unirnaviruses” and members of *Amalgaviridae*. However, viruses in both of these groups have nonsegmented genomes.    **Origin of the new family name:**  *Curvula*: from *Curvularia protuberata*, host of the first characterized virus representing the group | |

**Supporting evidence**

The proposal includes the following supporting datasets: Table 2 and Figures 1-4.

**Table 2.**  Sequence characteristics of proposed and probable members of the new virus family. Exemplar isolates of proposed *Curvulaviridae* members are in bold.

|  |  |  |  |  |  |  |  |  |
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| **Virus isolate** | **dsRNA1**  **(nt)** | **dsRNA2 (nt)** | **Accession numbers** | **Putative RdRp (aa)** | **dsRNA2**  **ORF1**  **(aa)** | **dsRNA2 ORF2 (aa)** | **Reference** | **Other information** |
| **Curvularia thermal tolerance virus** | 2149 | 1886 | EF120984 EF120985 | 371 | 331 | 168 | Márquez et al., 2007 | Complete genome  published |
| **Fusarium graminearum dsRNA mycovirus-4** | 2383 | 1739 | GQ140627 GQ140628 | 712 | 338 | 118 | Yu et al., 2009 | Complete genome  published |
| **Heterobasidion RNA virus 6** | 2050 | 1859 | HQ189459  MK468678 | 606 | 387 | 165 | Vainio et al., 2012 and unpublished | Complete genome  In GenBank |
| **Lactarius rufus RNA virus 1** | 2241 | 2049 | MT130415  MT130416 | 680 | 336 | 227 | Sutela & Vainio 2020 | Complete genome  published |
| **Lactarius tabidus RNA virus 1** | 2241 | 2049 | MG596784  MG596785 | 680 | 336 | 239 | Sutela & Vainio 2020 | Complete genome  published |
| **Rhizoctonia solani dsRNA virus 1** | 2379 | 1811 | JX976612  JX976613 | 692 | 465 | - | Zheng et al., 2013 | Complete genome  published |
| **Sclerotium hydrophilum virus 1** | 2121 | 1953 | NC\_030888  NC\_030891 | 624 | 381 | 135 | Wang et al., 2016 | Complete genome  published |
| **Trichoderma harzianum bipartite mycovirus 1** | 2088 | 1634 | MH536648  MH536649 | 631 | 314 | - | Liu et al., 2019 | Complete genome  published |
| Cryphonectria parasitica bipartite mycovirus 1 | 2026 | 1762 | KC549809  KC549810 | 592 | 331 | 145 | Unpublished | Complete genome in GenBank |
| Fusarium graminearum dsRNA mycovirus 5 | 2030 | 1740 | KX380787  KX380788 | 614 | 316 | 118 | Wang et al., 2017 | Complete genome  published |
| Gremmeniella abietina RNA virus 6 | 2165 | not reported | KJ742567 | 638 | - | - | Botella et al., 2015 | Complete dsRNA1 published |
| Myriodontium keratinophilum bipartite virus 1 | 2113  partial | 1915  partial | MG887758  MG887759 | 653 | 368  partial | 210 | Nerva et al., 2019 | Partial genome published |
| Penicillium aurantiogriseum bipartite virus 1 | 2110 partial | 1802 partial | KT601101  KT601102 | 614 | 322 | 166 | Nerva et al., 2016 | Complete coding sequences published |
| Rhizoctonia fumigata mycovirus 1 | 2311 | 1690 | KP209316  KP209317 | 706 | 485 | - | Unpublished | Complete genome  in GenBank |
| Rhizoctonia solani bipartite-like virus 1 | 1827  partial | 1888 | MK492913  MK492914 | 596 | 323 | 183 | Picarelli et al., 2019 | Partial genome published |

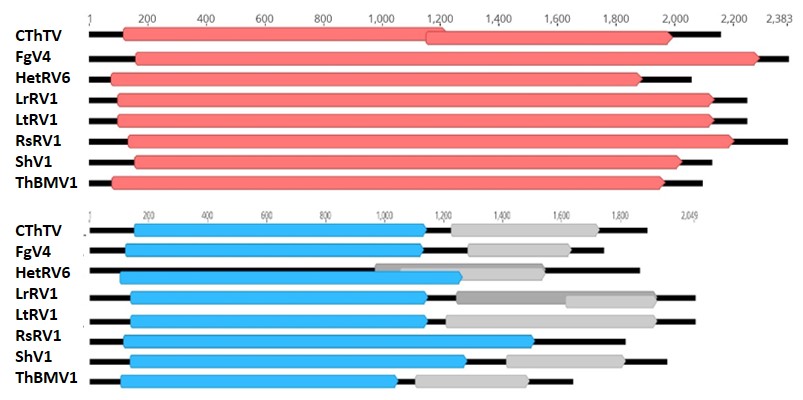


**dsRNA1** (~2.2 kb)

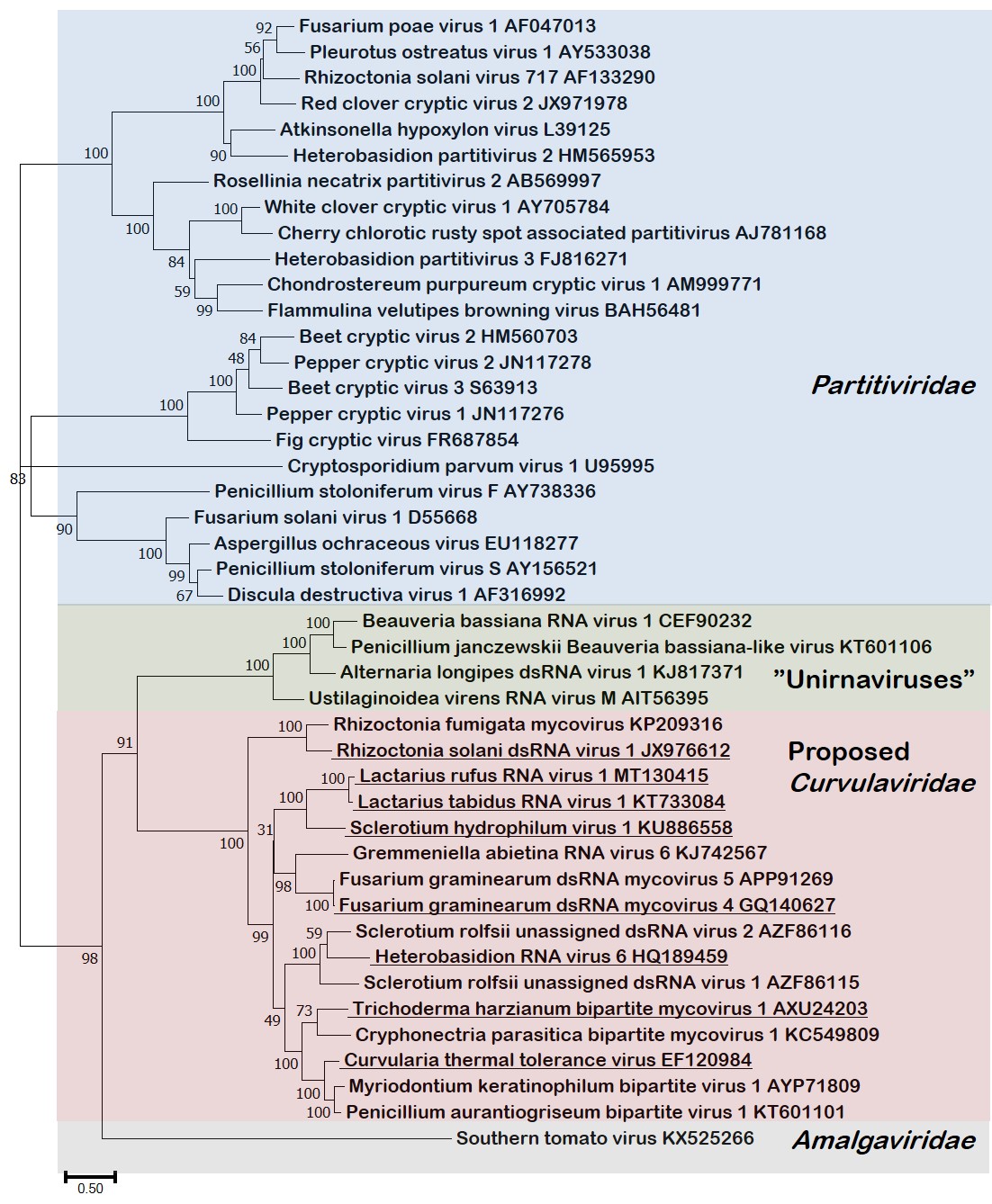
**dsRNA2** (~2.0 kb)

**rRNA**

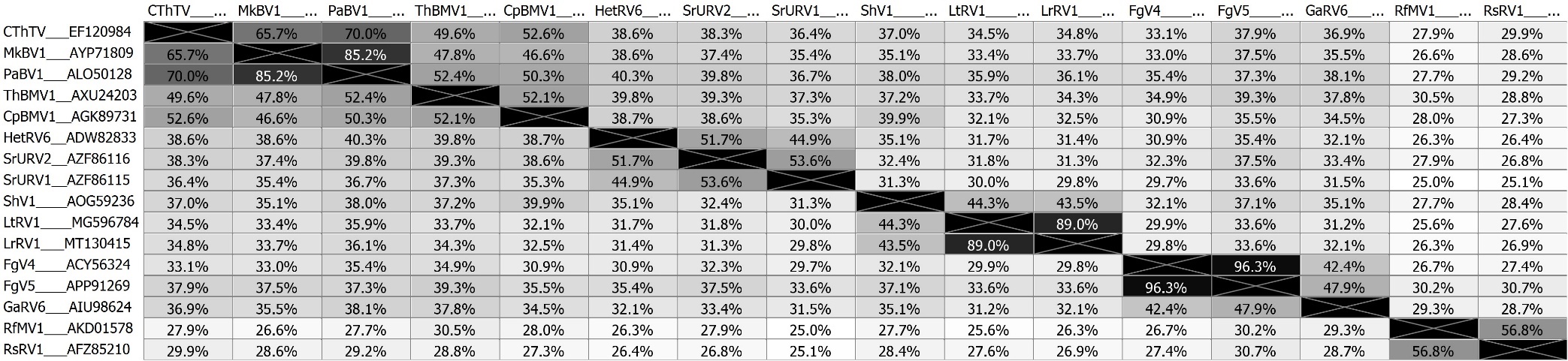
**Figure 1.** Agarose gel electrophoresis of dsRNA extracted from *Lactarius rufus* isolate Lr17-38I, showing the two genome segments of Lactarius rufus RNA virus 1

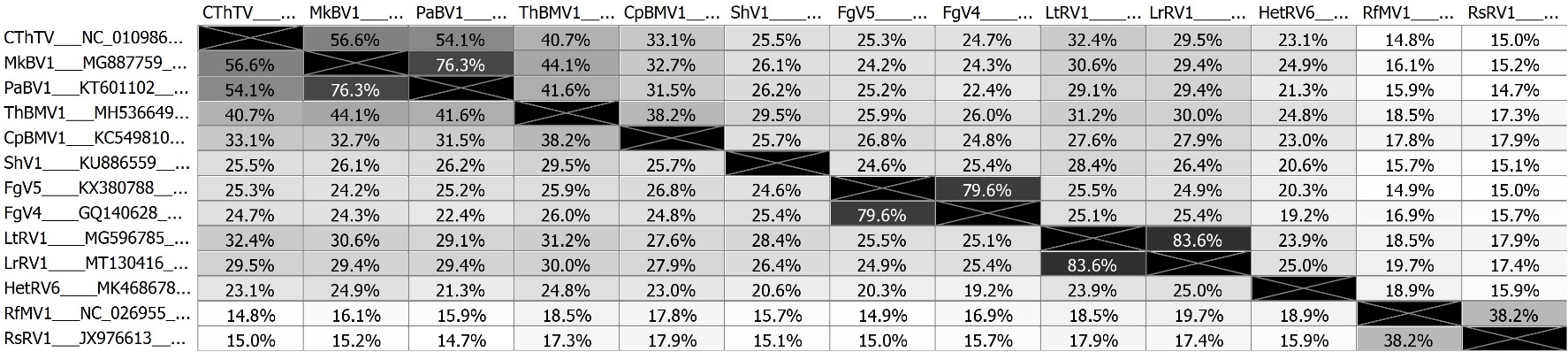


**Figure 2.** Genome organization of members of the proposed new species in 5’→3’ orientation.The upper panel shows dsRNA1 and lower panel dsRNA2 segments with predicted ORFs:dsRNA1 ORF1 predicted to encode an RdRp (red), dsRNA2 ORF1 with unknown function (blue). The second ORF of dsRNA2 is shown in light gray as predicted using AUG as a start codon. Alternative ORFs predicted using CUG as start codon are shown in dark gray.



**Figure 3.** Maximum Likelihood tree based on the complete amino acid sequences of predicted RdRps of members of the proposed family and related taxa. The evolutionary history was inferred using MEGA X (Kumar et al., 2018) using the LG model (LG+*G+I*) (Le and Gascuel, 2008). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches. Virus names and GenBank accession numbers are given; the proposed species are underlined.





**Figure 4.** Identity scores based on global pairwise alignments of the RdRp (upper heatmap) and a protein encoded by ORF1 of dsRNA2 (lower heatmap) of proposed and probable members of the new family. dsRNA2 sequences are not available for all isolates (see Table 1).

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