

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

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| **Code assigned:** | **2021.040M** |  |
| **Short title:** Create two new families (*Discoviridae*, *Tulasviridae*), each including one new genus (*Bunyavirales*) | | |
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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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| ICTV *Bunyavirales* Study Group |

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

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| No comments/dissent. |

**Submission dates**

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| Date first submitted to SC Chair | May 28, 2021 |
| Date of this revision (if different to above) | September 13, 2021 |

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

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| * In Figure 2, the multiple numbers were deemed confusing; please remove the branch lengths while keeping the bootstrap support numbers   Response: Done as requested.   * Also in Figure 2, bracket “*Hantaviridae*” should end with Laibin virus. Jonchet virus should be included in bracket “*Phasmaviridae*”.   Response: Fixed.   * Read the EC-distributed guidance on species naming document, confirm that proposed species names adhere to the guidance, and confirm that you would like to keep the proposed species names as originally proposed.   Response: Read, confirmed, and confirmed. |

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

**Name of accompanying Excel module**

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| 2021.040M.R.Bunyavirales\_2nfam\_2ngen\_6nsp |

**Abstract**

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| In recent years, a number of mycovirus RdRp-encoding sequences have been characterized from metatranscriptomic datasets and identified as likely members of the order *Bunyavirales*. Additional genomic data, such as associated nucleocapsid-encoding sequences, have rarely been identified and reported. Here we propose establishment of two new bunyaviral families to classify several viruses for which this information is available based on their genomic and phylogenetic features. |

**Text of proposal**

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| |  | | --- | | **NEW FAMILY: *Discoviridae***  A genomic sequence of a tri-segmented negative-sense bunyaviral-like RNA virus was originally generated from *Penicillium roseopurpureum* Dierckx, R.P. 1901 in a study of the virome of fungi of a sea cucumber, *Holothuria poli* Delle Chiaje, 1824 [2]. The three monocistronic genome segments encode a RNA-directed RNA polymerase (RdRp) (RNA1), a non-structural protein (Ns; RNA2), and a nucleocapsid (Nc; RNA3). The same virus, Penicillium roseopurpureum negative ssRNA virus 1 (PrNssV-1), was later discovered in a distantly related fungus (*Coniella diplodiella* (Speg.) Petr. & Syd., (1927)) [3]. Genome organization and phylogenetic analysis of the viral RdRp indicated that, albeit affinities to bunyavirals *sensu lato*, this virus cannot be assigned to established taxa in the order.  Sequences of a closely related virus from another fungus (*Penicillium atramentosum* Thom, C. 1910), named Penicillium discovirus (PDV), have been available from GenBank without associated publications. Finally, a number of related viruses with conserved Nc and Ns proteins was found in a dataset reporting the virome associated with lesions caused by an oomycete (*Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni, (1888)) [1]. Coding-complete sequences of three of these viruses are available: one of them clusters with PrNssV-1 and the others are distinct, but closely related and, in RdRp analysis, form a well-supported monophyletic clade distinct from those that include established bunyaviral taxa. Therefore, we propose the establishment of five new species in a new genus *Orthodiscovirus* in a new family *Discoviridae* to accommodate these viruses taxonomically. Some of the features of the viruses classified in the 5 new species are summarized in Table 1.  **Genome:**  The genomes of these viruses each comprise three segments of negative-sense RNA (Fig. 1). RNA1 of PrNssV-1, is ~6.5 kb long and encodes the putative RdRp including typical motifs of bunyaviral RdRps; RNA2 has a length of ~1.7 kb and encodes a putative Ns protein, and the third segment is ~1.2 kb long and encodes a putative Nc protein. The other viruses in the proposed family have similar genomic organization. The Nc and Ns proteins are conserved among all examined viruses in this clade. In the case of PDV, 3’ and 5’ RACE experiments were performed, revealed that the two termini of the three genomic segments shared a conserved motif (5’-ACACAAAGCACCCC3’-) that enables panhandle pairing of the 5’ and 3’ ends of each genomic segment [5].  **Phylogenetic relationships**:  A phylogenetic tree of the order *Bunyavirales* (Fig. 2) demonstrates the monophyletic clade. A MAFFT alignment/percentage identity matrix is shown in Fig. 3. This matrix confirms that some identified viruses belong to the same species as they share amino acid content exceeding demarcation threshold of 80% identity in the RdRp among members of distinct proposed species.  Etymologies of newly proposed taxa: See Excel module.  **NEW FAMILY: *Tulasviridae***  Characterization of an orchid mycorrhizal virome resulted in discovery of a virus named Tulasella bunyavirales-like virus 1 (TB-LV) with features unprecedented in other members of the order *Bunyavirales* [4]. This virus has a genome composed of a single large molecule of ambisense RNA encoding three distinct ORFs. Two of these ORFs encode proteins with motifs that allow to assign a putative function: the product of the largest ORF is a putative RdRp, whereas the shortest one, in opposite orientation, encodes a putative Nc. The RdRp, although quite distant from those currently present in databases, aligns with other bunyaviral RdRps. For this reason, we propose to place TB-LV in a new genus (*Orthotulasvirus*) in a new family (*Tulasviridae*).  **Genome:**  The genome organization of TB-LV comprises a single molecule of negative sense RNA of ~12 kb encoding three ORFs (Fig. 4). The largest ORF, in negative sense orientation, encodes the putative RdRp (pfam 04196) with a calculated molecular mass of 290.3 kDa. The shortest ORF, in the opposite orientation, encodes the putative Nc with a calculated molecular mass of 27.8 kDa (PF02477). Finally, a third ORF, upstream of the RdRp-encoding ORF and in the same orientation encodes a putative protein of 129.3 kDa, without detectable similarity to any protein in databases. The 3’ and 5’ end of the genome segment are, as far as known, not complementary.  **Phylogenetic relationships**:  The phylogenetic analysis and identity matrix based on an RdRp alignment with representatives of current bunyaviral families (Fig. 2 and Fig. 3), show that this virus belongs to a distinct lineage in the order and should be classified in a new species to be placed in a newly created genus and family (*Orthotulasvirus*: *Tulasviridae*).  Less than 80% identity in the amino-acid content of the whole RdRp among members of distinct species is proposed as a demarcation threshold in the genus *Orthotulasvirus*.  Etymologies of newly proposed taxa: See Excel module. | |

**Supporting evidence**

**Table 1** Seven viruses accommodated in 6newly proposed species of the newly established families *Discoviridae* and *Tulasviridae*

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| --- | --- | --- | --- | --- | --- |
| New family | New species | Newly identified virus | Accession number(P/N) | Host | Reference |
| *Discoviridae* | *Orthodiscovirus penicillii* | Penicillium roseopurpureum negative ssRNA virus 1 | MN163273; MN163274; MG887749 | *Penicillium roseopurpureum* | 2 |
|  | *Orthodiscovirus missouriense* | Penicillium discovirus | MF142460; MF142459; MF142458 | *Penicillium atramentosum* | 5 |
|  | *Orthodiscovirus coniellae* | Coniothyrium diplodiella negative-stranded RNA virus 1 | MN532680; MN532679; MK584854 | *Coniella diplodiella* | 1 |
|  |  | Plasmopara viticola lesion associated mycobunyavirales-like virus 9 | MT258649;  MT258648;  MN585282 | Associated with *Plasmopara viticola* lesions | 1 |
|  | *Orthodiscovirus hispaniae* | Plasmopara viticola lesion associated mycobunyavirales-like virus 8 | MT188509; MT188506; MN585278 | Associated with *Plasmopara viticola* lesions | 1 |
|  | *Orthodiscovirus iberiae* | Plasmopara viticola lesion associated mycobunyavirales-like virus 4 | MT188508; MT188505; MN548097 | Associated with *Plasmopara viticola* lesions | 1 |
| *Tulasviridae* | *Orthotulasvirus tulasnellae* | Tulasnella bunyavirales-like virus 1 | MN793997 | *Tulasnella* spp. | 4 |

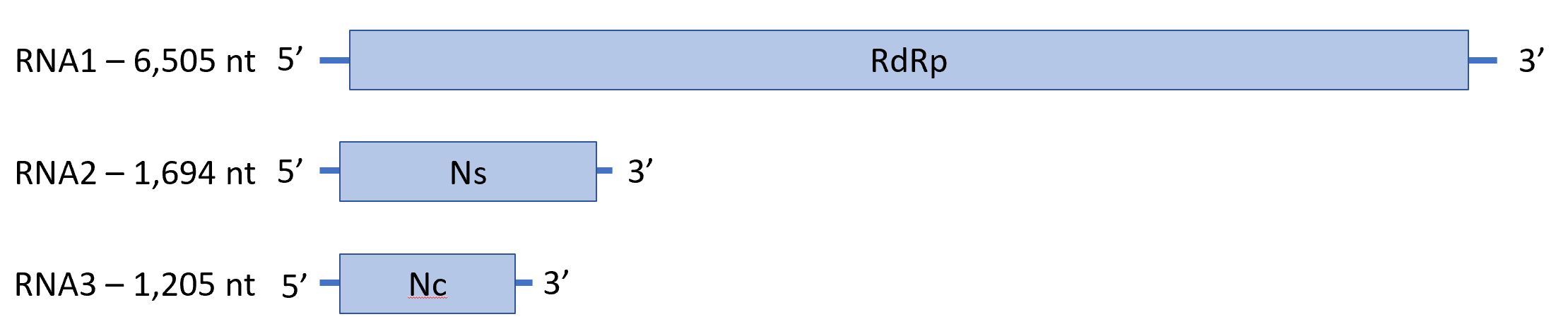
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Figure 1: Genome organization of a representative virus, Penicillium roseopurpureum negative sense RNA virus 1, belonging to the proposed genus *Orthodiscovirus* in the proposed family *Discoviridae*. The main ORFs encoded by each genomic RNA are represented by rectangles. RdRp=RNA-directed RNA polymerase; Ns=nonstructural protein; Nc=nucleocapsid protein

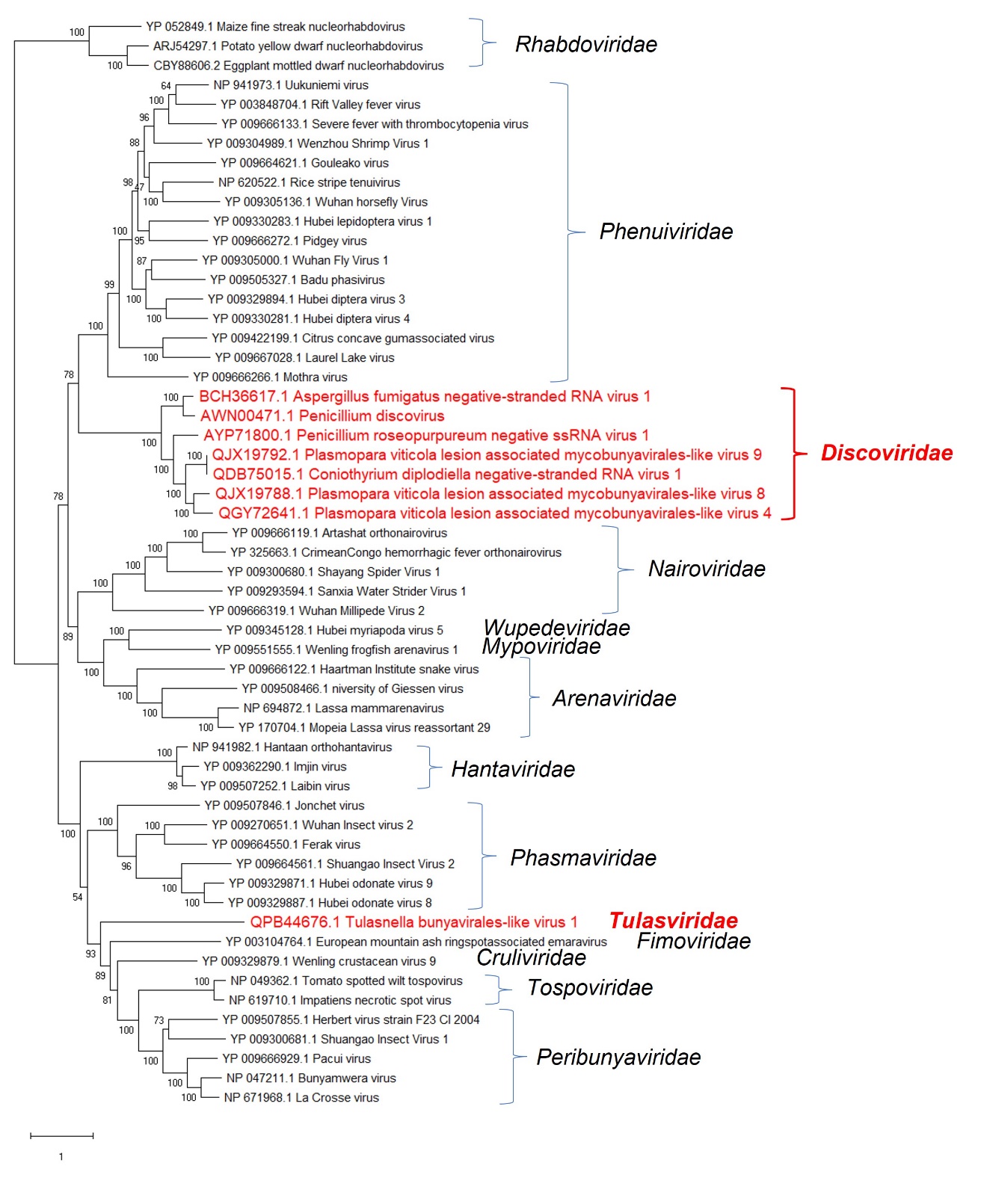
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Figure 2: Phylogenetic analysis of aligned RNA-directed RNA polymerase sequences of viruses belonging to the order *Bunyavirales*: the main families are represented by one or more virus sequences. Newly proposed families are indicated in red. MAFFT software was used to align the sequences, whereas the tree was derived with the Maximum Likelihood methodology implemented with IQ-TREE.

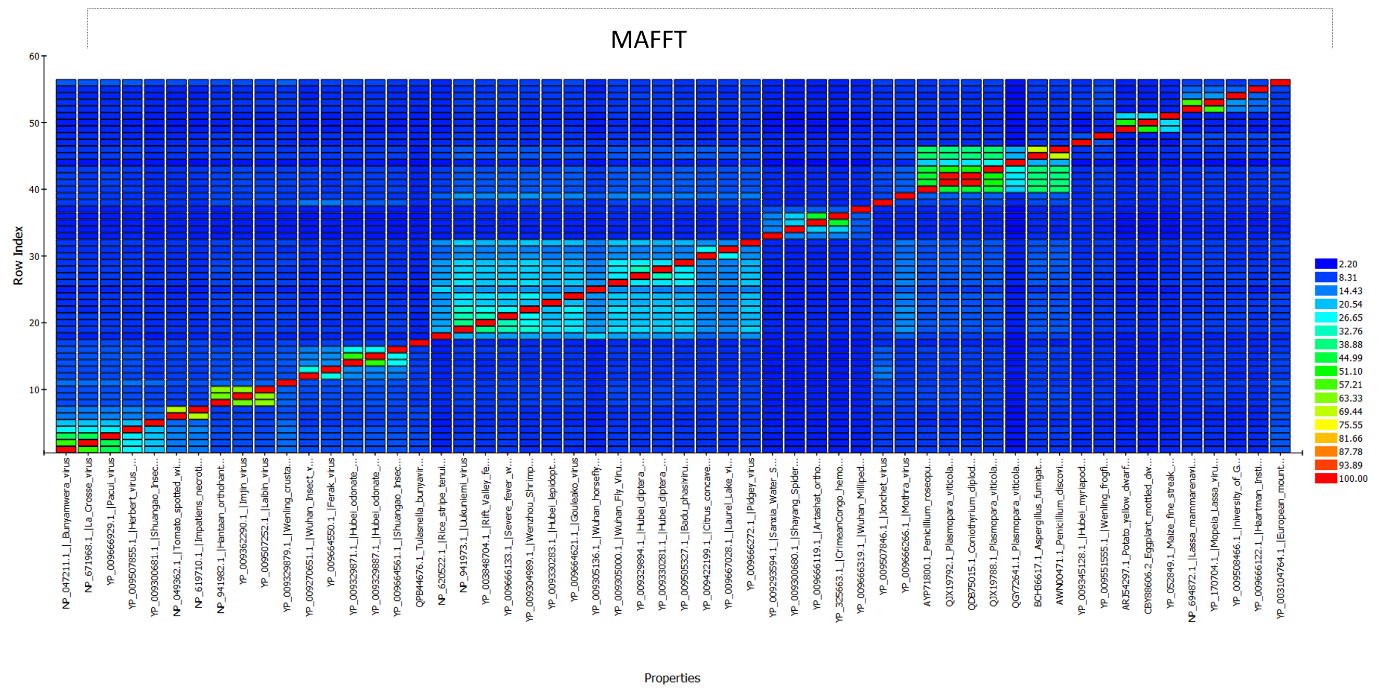


Figure 3: Heatmap of a table representing the pairwise identity percentages derived from alignments of bunyaviral RdRps.

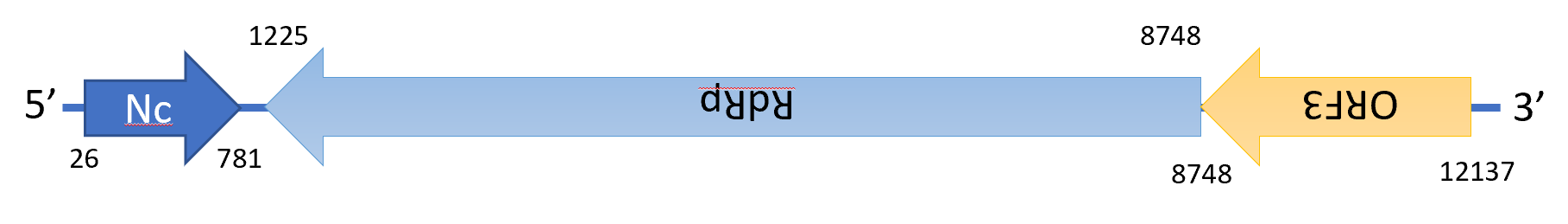
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Figure 4: Genome organization of a Tulasnella bunyavirales-like virus 1, belonging to the proposed genus *Orthotulasvirus* in the proposed family *Tulasviridae*. The main ORFs of the genomic RNA are represented by arrows. RdRp=RNA-directed RNA polymerase; Nc=nucleocapsid; ORF3=open reading frame 3

**References**

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5. Krishnamurthy SR (2017) The reduction of viral dark matter by expanding of viral diversity. PhD dissertation, Washington University, Saint Louis, Missouri, USA. [Expansion of Microbial Virology by Impetus of the Reduction of Viral Dark Matter (wustl.edu)](https://openscholarship.wustl.edu/cgi/viewcontent.cgi?article=2253&context=art_sci_etds)