

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

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| **Code assigned:** | **2020.004P** |  |
| **Short title:** Create three new species in the genera *Closterovirus*, *Ampleovirus* and *Velarivirus* (*Martellivirales*: *Closteroviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| Family *Closteroviridae* |

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

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

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**Submission dates**

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| Date first submitted to SC Chair | July 28, 2020 |
| Date of this revision (if different to above) |  |

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

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**Part 2:** **NON-TAXONOMIC PROPOSAL**

**Text of proposal**

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

**Name of accompanying Excel module**

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| 2020.004P.R.Closteroviridae\_3nsp.xlsx |

**Abstract**

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| This taxonomic proposal considers a previously characterized virus (carrot closterovirus 1, CtCV1)as a representative of a new species in the genus *Closterovirus,* a recently reported virus (Malus domestica virus A, MdoVA) as a member of a new species in the genus *Velarivirus*,and a recently identified virus (yam asymptomatic virus 1, YaV1) as a prototype of a new species in the genus *Ampelovirus* based on the following species demarcation criteria: size of their single-stranded RNA genome (15,923 nt for CtCV1,17,003 nt for MdoVA and 14,885 nt for YaV1), overall low genetic relatedness with most members of the family *Closteroviridae*, mass of the coat protein (23KDa for CtCV1, 37kDa for MdoVA and 36kDa for YaV1) and less than 75% amino acid sequence identity in the three taxonomically relevant open reading frames, i.e. RNA-dependent RNA polymerase (RdRP), heat shock protein 70 homologue (HSP70h) and coat protein CP), with their closest relative within the family *Closteroviridae*. |

**Text of proposal**

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Ampeloviruses are transmitted by pseudococcid mealybugs and soft scale insects, closteroviruses are transmitted by aphids, and criniviruses are transmitted by whiteflies. No vectors are known for velariviruses. Species demarcation criteria used for all genera in the family *Closteroviridae* are particle size, size of the coat protein, genome structure and organization [number and relative location of open reading frames (ORFs)], vector species and specificity, cytopathological features, host range, as well as amino acid sequence of relevant gene products, i.e. RNA-dependent RNA polymerase (RdRp), coat protein (CP), heat shock protein 70 homolog (HSP70h), differing by more than 25% among viruses belonging to distinct species.  In this taxonomic proposal, we suggest the creation of three new species in the family, one in each of the genera *Ampelovirus*, *Closterovirus* and *Velarivirus*, and the classification of these three recently characterized viruses with a genome organization resembling that of recognized members of these three taxa within the family *Closteroviridae*.  **Malus domestica virus A (MdoVA), a member of a new species of the genus *Velarivirus* in the family *Closteroviridae*.**  A novel virus was identified from apple (*Malus domestica*) in the Czech Republic (Koloniuk et al. 2020). Total RNA was isolated from an unknown apple cultivar exhibiting foliar light green mosaic symptoms and used to prepare an RNA library after ribosomal RNA depletion for high throughput sequencing. More than 120 million reads were obtained and contigs were assembled. A BLASTn analysis yielded hits against three known apple viruses (apple chlorotic leaf spot virus apple green crinkle-associated virus and apple mosaic virus) and one ~15kb long contig had substantial amino acid identity with little cherry virus 1 (LChV-1) from the genus *Velarivirus* in the family *Closteroviridae*. The long viral contig sequence was tentatively attributed to a virus named Malus domestica virus A (MdoVA). The primary structure of the 5’ and 3’ termini of the MdoVA genome was determined by RACE and specific primers. The entire genome was verified by Sanger sequencing. The complete genome of MdoVA isolate J1 is 17,003 nt long (GenBank accession number MN548734) and contains nine putative open reading frames (ORF) (Figure 1). It is the longest known genome for any velarivirus. The 5’ and 3’ noncoding regions are 81 nt and 227 nt long, respectively. ORF1a encodes a replication-associated protein with conserved domains for a methyltransferase and a helicase. ORF1b is presumably expressed by a +1 ribosomal frameshift and encodes an RNA dependent RNA polymerase (RdRP). ORF2 encodes a small protein p4 with a transmembrane domain. ORF3 encodes a homologue of the heat shock protein 70 (HSP70h). ORF4 is predicted to overlap with ORF3 and encode a protein p10 of unknown function. ORF5 encodes the putative coat protein (CP) and ORF6 encodes the minor capsid (CPm). ORF8 and ORF9 encode proteins p27 and p28, respectively (Figure 1). Phylogenetic analyses showed that MdoVA clusters with members of the genus *Velarivirus* in the family *Closteroviridae* (Figure 2). Sequence comparisons for the taxonomically relevant RdRP, HSP70h and CP sequences revealed MdoVA is most closely related to LChV-1, with amino acid sequence identity values of 63, 44 and 26%, respectively (Table 1). MdoVA was detected in one out of 67 apple trees by RT-PCR using specific primers. Taken together, MdoVA is distinct from any currently recognized member of the family *Closteroviridae*, showing less than 75% amino acid sequence identity in the RdRP, HSP70h and CP. We propose to recognize MdoVA-J1 as a member of new species in the genus *Velarivirus* of the family *Closteroviridae* for which the name *Malus domestica virus A* is proposed.  **Table 1.** Genetic relatedness of Malus domestica virus A (MdoVA), proposed member of a new virus species, with little cherry virus 1 (LChV-1), its closest relative.   |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | | **Acronym** | **Virus Name** | **Most closely related virus** | **% CP aa sequence identity** | **% RdRp aa sequence identity** | **% HSP70h aa sequence identity** | | MdoVA | Malus domestica virus A | little cherry virus 1 | 26 | 63 | 44 |   **Carrot closterovirus 1 (CtCV1), a member of a new species of the genus *Closterovirus* in the family *Closteroviridae*.**  A previously characterized virus was identified from carrot (*Daucus carota* subsp. *sativus*)in the United Kingdom (Adams et al. 2014). Total RNA was extracted from asymptomatic carrot samples and carrot samples showing internal necrosis and used for library constructions and sequencing on an Illumina platform. After trimming, sequences were compared to the GenBank protein database and viral reads were extracted. In addition to carrot yellow leaf virus (CYLV), carrot red leaf virus (CtrLV), carrot red leaf-associated RNA (CtRLVaRNA), beet western yellows virus-associated RNA (BWY-VaRNA) carrot mottle virus (CMoV), carrot torradovirus 1 (CTV1), carrot chordovirus 1 (CtChV1) carrot chordovirus 2 (CtChV1), a new virus tentatively named carrot closterovirus 1 (CtCV1) was identified. The complete coding sequence of CtCV1 isolate CUCV\_S8 is 15,923 nt long (GenBank Accession number KF533697) and contains eight open reading frames (Figure 1). The 5’ and 3’ terminal sequences of the CtCV1 genome were not determined. ORF1a encodes a replication-associated protein with conserved domains for a papain-like protease, a methyltransferase and a helicase. ORF1b is likely expressed by a +1 ribosomal frameshift and encodes an RNA dependent RNA polymerase (RdRP). ORF2 encodes a p7 protein, ORF3 encodes a heat shock protein 70 homologue (HSP70h), ORF4 a p60 protein, ORF5 a minor coat protein (CPm) and ORF6 the coat protein (CP). ORF7 encodes a p22 protein and ORF8 a p23 protein with conserved domains of viral RNA silencing suppressors (Figure 1). Phylogenetic analyses showed that CtCV1 clusters with members of the genus *Closterovirus* in the family *Closteroviridae* (Figure 2). Sequence comparisons for the taxonomically relevant RdRP, HSP70h and CP sequences revealed CtCV1 is most closely related to CYLV with amino acid sequence identity values of 84, 64 and 46%, respectively (Table 2). The occurrence of CtCV-1 was documented in four out of 201 carrot samples tested by RT-qPCR using specific primers. No information is available on potential vectors of CtCV1. Taken together, CtCV1 is distinct from any currently recognized member of the family *Closteroviridae*, showing less than 75% amino acid sequence identity in the HSP70h and CP. We propose to create a novel species in the genus *Closterovirus*, *Carrot closterovirus 1*, typified by CtCV1 isolate CUCV\_S8.  **Table 2.** Genetic relatedness of carrot closterovirus 1 (CtCV1), member of a proposed novel homonymous species, with carrot yellow leaf virus (CYLV), its closest relative.   |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | | **Acronym** | **Virus Name** | **Most closely related virus** | **% CP aa sequence identity** | **% RdRp aa sequence identity** | **% HSP70h aa sequence identity** | | CtCV1 | carrot closterovirus 1 | carrot yellow leaf virus | 46 | 84 | 64 |   **Yam asymptomatic virus 1 (YaV1), a member of new species in the genus *Ampelovirus* in the family *Closteroviridae****.*  A novel virus was recently identified from yam (*Dioscorea* *alata*) in France (Marais et al. 2020). dsRNA was isolated from leaves of yam accessions maintained in a germplasm repository and cDNA libraries were generated for sequencing using an Illumina HiSeq 2000 system. Sequence contigs with similarity to viruses were selected following BLASTn and BLASTx analyses performed against publicly available non-redundant nucleotide or protein databases. Sequencing of dsRNAs from *D. alata* accession VU567Da originally collected in Vanuatu in 2003 and maintained in a collection in Montpellier, France generated 930,548 paired reads from which the near complete genome of a novel virus tentatively named yam asymptomatic virus 1 (YaV1) was assembled. The 5’ and 3’ end of the YaV1 genome were amplified by RACE and Sanger sequenced. The complete nucleotide sequence of the novel virus is 14,855 nt long (GenBank accession number (MT409627). The 5’ and 3’ noncoding extremities are 61 nt and 192 nt long, respectively. The YaV1 genome has ten putative (ORFs) (Figure 1) and an organization similar to that of little cherry virus 2 (LChV2) from subgroup 1 of the genus *Ampelovirus* in the family *Closteroviridae.* The first ORF (ORF0) from the 5’ end of the YaV1 genome is in the negative orientation and encodes a putative 17.7 kDa protein (p17) with no similarity to protein sequences publicly available in databases. ORF1a is a first of nine ORFs in the positive sense and encodes a putative 179 kDa protein with catalytic domains of a methyltransferase at aa positions 80-294 and a helicase at aa positions 1297-1557. ORF1b putatively expressed as a frameshift fusion to ORF1a encodes a p60 protein with the catalytic domain of an RNA-dependent RNA polymerase at aa positions 69-511. ORF2 encodes a putative 54.5 kDa protein with domains of the coat protein. ORF3 encodes a small putative 6.5 kDa hydrophobic protein, while ORF4 encodes a putative 59.8 kDa protein with HSP70h motifs at aa positions 3-433. ORF5 encodes a putative 54.9 kDa protein. ORF6 encodes a putative 21.9 kDa protein with an unknown function. ORF7 encodes a putative 36.2 kDa protein with a coat protein (CP) motif at aa positions 191-314 and ORF8 codes a putative 22.6 kDa protein with no conserved motifs (Figure 1). Phylogenetic analyses confirmed that YaV1 clusters with strong support with members of the genus *Ampelovirus* in the family *Closteroviridae* (Figure 2). Sequence comparisons for the taxonomically relevant RdRP, HSP70h and CP sequences revealed YaV1 is most closely related to little cherry virus 2 (LChV2) with amino acid sequence identity values of 70.5, 61.1 and 57.3%, respectively (Table 3). The occurrence of YaV1 was documented in 83 out of 170 yam samples from a germplasm collection tested by RT-PCR using specific primers. When found as a single infection in yam, YaV1 did not cause any disease symptoms. No information is available on potential vectors of YaV1. Taken together, YaV1 is distinct from any currently recognized member of the family *Closteroviridae*, showing less than 75% amino acid sequence identity in the RdRP, HSP70h and CP. We propose to create a new species in the genus *Ampelovirus,* denominated *Yam asymptomatic virus 1*, to accommodate recently characterized yam asymptomatic virus 1 (YaV1), isolate VU567Da.  **Table 3.** Genetic relatedness of yam asymptomatic virus (YaV1), member of proposed new virus species, with little cherry virus 2 (LCh2), its closest relative.   |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | | **Acronym** | **Name** | **Most closely related virus** | **% CP aa sequence identity** | **% RdRp aa sequence identity** | **% HSP70h aa sequence identity** | | YaV1 | Yam asymptomatic virus 1 | little cherry virus 2 | 57.3 | 70.5 | 61.1 | | |

**Supporting evidence**

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**Figure 1.** Schematic representation of the genome organization of Malus domestica virus A (MdoVA) (panel A), yam asymptomatic virus 1 (YaV1) (panel B), carrot closterovirus 1 (CtCV1) (panel C) and representative members of four genera within the family *Closteroviridae* (panel D), including beet yellows virus (BYV) from the genus *Closterovirus*, grapevine leafroll-associated virus 3 (GLRaV-3) from the genus *Ampelovirus*, Cordyline virus 1 (CoV1) from the genus *Velarivirus*, and lettuce infectious yellows virus (LIYV) from the genus *Crinivirus*. Blocks represent predicted open reading frames (ORFs). The replicase proteins are shown in yellow with the papain-like protease (Pro), methyltransferase (Met), alkB domain (AlkB), helicase (HEL), and RNA-dependent RNA polymerase (RdRP) domains. Small transmembrane proteins (p4, p5 and/or p6) are shown in grey, the heat shock protein 70 homolog (HSP70h) in light green, the coat protein (CP) in light purple, and the minor coat protein (CPm) in pink.

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**Figure 2.** Maximum likelihood phylogenetic tree showing the relationships between Malus domestica virus A (MdoVA), carrot closterovirus 1 (CtCV1) and yam asymptomatic virus 1 (YaV1) with recognized members of the family *Closteroviridae* based on an alignment of the complete amino acid sequence of the heat shock protein 70 homolog using MUSCLE (Edgar 2004). The maximum likelihood tree was inferred using RAxML (Stamatakis 2014) in the T-REX web server (Boc et al., 2012). Distances are proportional to branch lengths and the bar represents the genetic distance. The heat shock protein 70 from *Arabidopsis thaliana* (AEE75218) was used as outgroup. The sequence of MdoVA in the genus *Velarivirus* is boxed in blue and the sequence of CtCV1 in the genus *Closterovirus* is boxed in green. The GenBank accession number used for each virus is as follows: actinidia virus 1 (AcV-1, KX857665), air potato ampelovirus 1 (AiPoV-1, MH206615), areca palm velarivirus 1 (ArPV1, KR349464), arracacha virus 1 (AV-1, MG919988), bean yellow disorder virus (BYDV, EU191904), beet pseudoyellows virus (BPYV, AY330918), beet yellow stunt virus (BYSV, U51931), beet yellows virus (BYV, AF056575), blackcurrant closterovirus 1 (BCCV1, MH267701), blackberry vein banding-associated virus (BVBaV, KC904540), blackberry yellow vein-associated virus (BYVaV, AY776335), blueberry virus A (BVA, AB733585), carnation necrotic fleck virus (CNFV, GU234166), carrot closterovirus 1 (CtCV1, KF533697), carrot yellow leaf virus (CYLV, FJ869862), citrus tristeza virus (CTV, U16304), Cordyline virus 1 (CoV-1, HM588723), Cordyline virus 2 (CoV-2, JQ599282), Cordyline virus 3 (CoV-3, JQ599283), Cordyline virus 4 (CoV-4, JQ599284), cucurbit yellow stunting disorder virus (CYSDV, AY242077), diodia vein chlorosis virus (DVCV, CQ376201), fig leaf mottle-associated virus 2 (FLMaV-2, FJ473383), fig mild mottle-associated virus (FMMaV, FJ611959), grapevine leafroll-associated virus 1 (GLRaV-1, JQ023131), grapevine leafroll-associated virus 2 (GLRaV-2, JX513891), grapevine leafroll-associated virus 3 (GLRaV-3, EU259806), grapevine leafroll-associated virus 4 (GLRaV-4, FJ467503), grapevine leafroll-associated virus 7 (GLRaV-7, HE588185), grapevine leafroll-associated virus 13 (GLRaV-13, LC052212), lettuce chlorosis virus (LCV, FJ380118), lettuce infectious yellows virus (LIYV, U15440), little cherry virus 1 (LChV-1, EU715989), little cherry virus 2 (LChV-2, AF531505), Malus domestica virus A (MdoVA, MN548734), mint vein banding-associated virus (MVBaV, KJ572575), mint virus 1 (MV-1, AY792620), persimmon virus B (PeBV, AB923924), pineapple mealybug wilt-associated 1 (PMWaV-1, AF414119), pineapple mealybug wilt-associated 2 (PMWaV-2, AF283103), pineapple mealybug wilt-associated 3 (PMWaV-3, DQ399259), pistachio ampelovirus A (PAVA, MF198462), plum bark necrosis stem pitting-associated virus (PBNSPaV, EF546442), raspberry leaf mottle virus (RLMoV, DQ357218), Rehmannia virus1 (ReV-1, MH033657), rose leaf rosette-associated virus (RLRaV, KJ7488003), strawberry chlorotic fleck-associated virus (SCFaV, DQ860839), potato yellow vein virus (PYVV, AJ557128), strawberry pallidosis-associated virus (SPaV, AY488138), sweet potato chlorotic stunt virus (SPCSV, AJ428554), tetterwort vein chlorosis virus (TwVSV, KR002687), tobacco virus 1 (TV1, KT203917), tomato chlorosis virus (ToCV, AY903447), tomato infectious chlorosis virus (TICV, FJ815440) and yam asymptomatic virus 1 (YaV1, MT409627).

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