

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

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| **Code assigned:** | **2020.032P** |  |
| **Short title:** Create one new species in the subgenus *Mandarivirus*, genus *Potexvirus* (*Tymovirales*: *Alphaflexiviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Alphaflexiviridae* Study Group |

**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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| **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 | June 30, 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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| 2020.032P.R.Mandarivirus\_1nsp.xlxs |

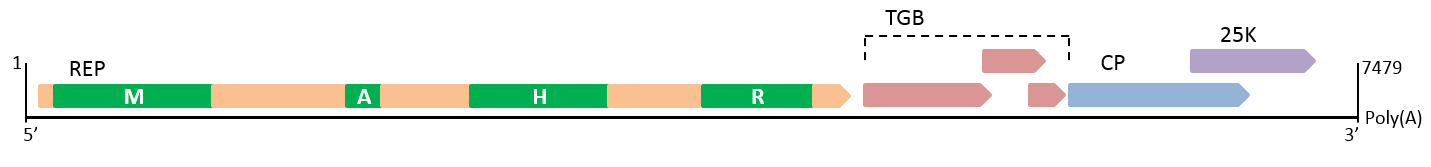
**Abstract**

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| A novel virus, named citrus yellow mottle-associated virus (CiYMaV), was discovered from one symptomatic citrus tree by igh-throughput sequencing (Wu et al. 2020). Genome features of CiYMaV are typical of members of the subgenus *Mandarivirus* (genus *Potexvirus*, family *Alphaflexiviridae*). The proteins encoded by CiYMaV share high sequence identity, conserved motifs and phylogenetic relationships with the corresponding proteins encoded by Indian citrus ringspot virus (ICRSV) and citrus yellow vein clearing virus (CYVCV), the two current members of the subgenus *Mandarivirus.* Virus particles of CiYMaV with elongated flexuous shape and size resembling those of mandariviruses were observed by transmission electron microscopy. Moreover, CYVCV is the most closely related virus to CiYMaV, the two viruses can be serologically and biologically discriminated from each other. Therefore, we suggest that CiYMaV should be classified as a novel species in the subgenus *Mandarivirus*. |

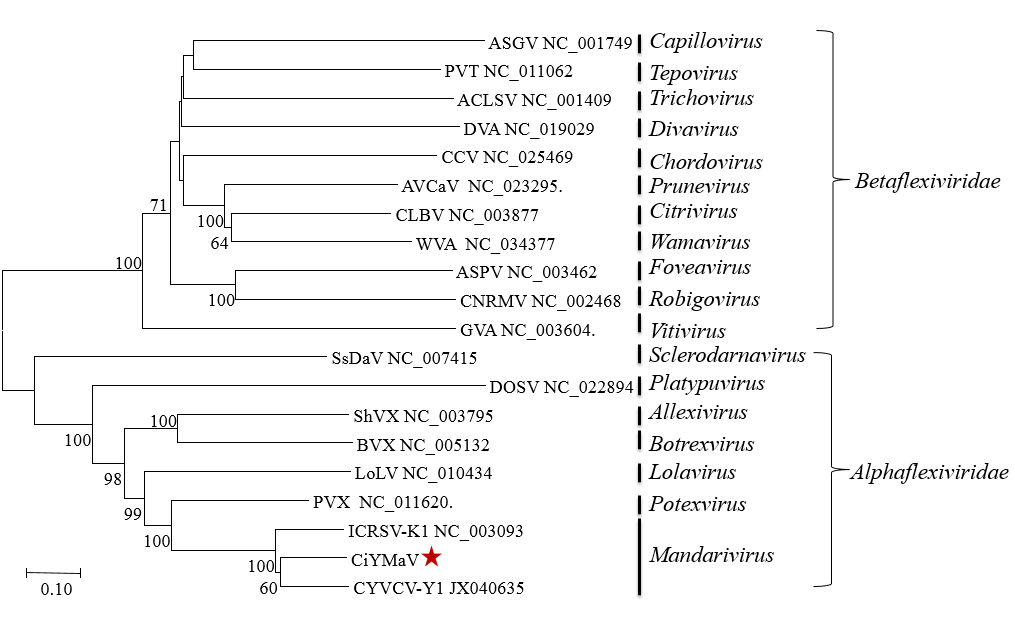
**Text of proposal**

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| --- | --- |
| |  | | --- | | The molecular, phylogenetic, biological, ultrastructural and serological features of CiYMaV support the proposal of classifying it as a novel species in the genus *Madarivirus*:   1. The genome of CiYMaV (Accession number MK957246) is composed of a positive-sense single-stranded RNA of 7,479 nt, with six open reading frames (ORFs), typically of members of the subgenus *Mandarivirus* (family *Alphaflexiviridae*) (Fig. 1) (Loconsole et al. 2012, Rustici et al. 2002). CiYMaV shares 75% aa identity of the CP with members of the subgenus *Mandarivirus* (Table 1), that satisfies the current species demarcation criteria for the subgenus *Mandarivirus* listed in the ICTV 10th Report; 2. Phylogenetic trees reconstructed with nucleotide sequences of the whole genome (Fig. 2A), amino acid sequences of the CP (Fig. 2B) and REP (Fig. 2C) of representative members of the families *Alphaflexividae*, *Betaflexiviridae* andCiYMaV,showed that CiYMaV and the two mandariviruses (CYVCV and ICRSV) clustered into a single clader; 3. Virus particles of CiYMaV with elongated flexuous shape and size resembling those of mandariviruses was observed by transmission electron microscopy (Fig. 3) (Loconsole et al. 2012, Rustici et al. 2000); 4. The relationship between CYVCV and CiYMaV. The observed cross-reactivity of a CYVCV-specific antibody (MAB 1E1) with CiYMaV and the absence of any reactivity of CiYMaV with another CYVCV-specific MAB (MAB 26A1) (Fig. 4B). CYVCV induced vein clearing in Daidai sour orange (Fig. 5A) and Eureka lemon (Fig. 5B), while CiYMaV was not associated with any leaf symptom in these hosts (Fig. 5E-F) but with vein yellowing and mottling in Symons sweet orange (Fig. 5G), whereas CYVCV did not induce any leaf symptom in this host (Fig. 5C). | |

**Supporting evidence**



**Figure 1.** Genome structure of citrus yellow mottle-associated virus (CiYMaV). Open reading frames and conserved domains were identified using conserved domain database search at NCBI. REP, replication-associated polyproteins; M, viral methyltransferase domain; A, AlkB-like domain; H, viral helicase domain; R, RNA-dependent RNA polymerase 2 domain; TGB, triple gene block; CP, capsid protein; 25K, 25K-like putative protein of 25.3KDa.

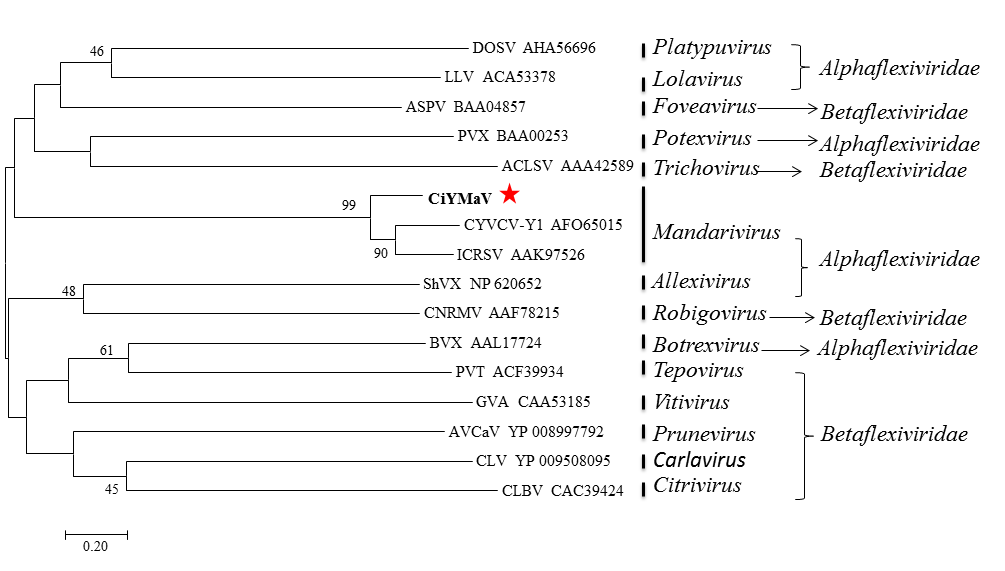


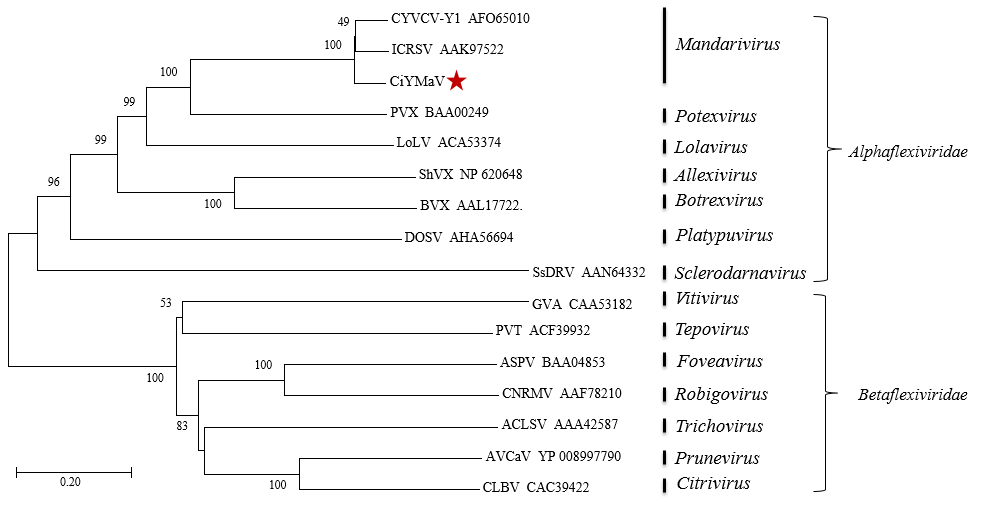
**A**

**Figure 2.** Phylogenetic analyses of the whole genome nucleotide of citrus yellow mottle-associated virus (CiYMaV) (**A**), amino acid sequences of the CP (**B**) and REP (**C**) gene encoded by CiYMaV, and the genome, CP and REP of representative members of the families *Alphaflexividae* and *Betaflexiviridae*. Viruses included in the analyses are citrus yellow vein clearing virus (CYVCV), Indian citrus ringspot virus (ICRSV), shallot virus X (ShVX), botrytis virus X (BVX), lolium latent virus (LoLV), donkey orchid symptomless virus (DOSV), potato virus X (PVX), sclerotinia sclerotiorum debilitation-associated RNA virus (SsDRV), apple stem pitting virus (ASPV), cherry necrotic rusty mottle virus (CNRMV), citrus leaf blotch virus (CLBV), apricot vein clearing associated virus (AVCaV), potato virus T (PVT), apple chlorotic leaf spot virus (ACLSV), grapevine virus A (GVA), watermelon virus A (WVA), carrot Ch virus 1 (CCV-1). The phylogenetic trees were inferred by neighbor-joining method, using 1000 bootstrap replications, with the values lower than 45% not shown.

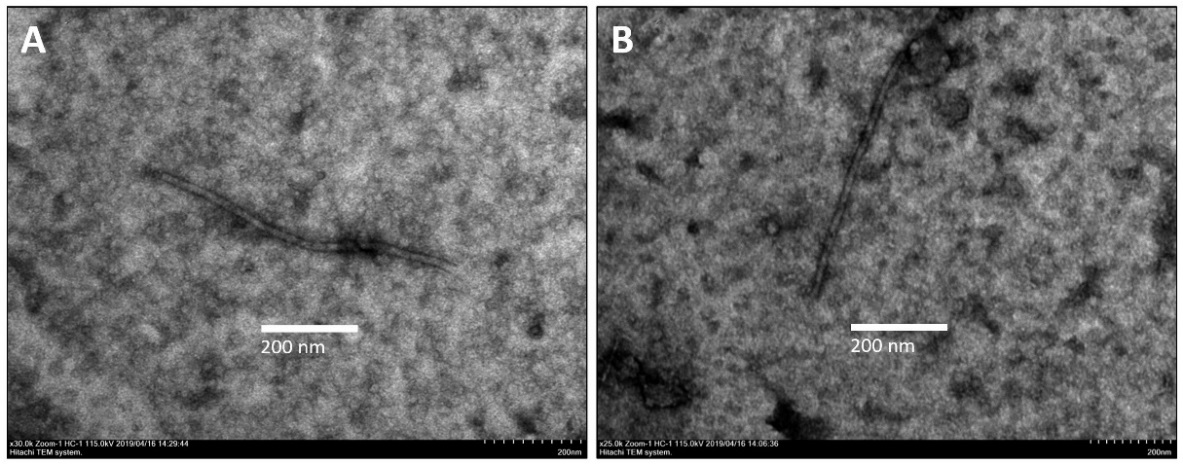
**Figure 2 (cont.)**

**B**

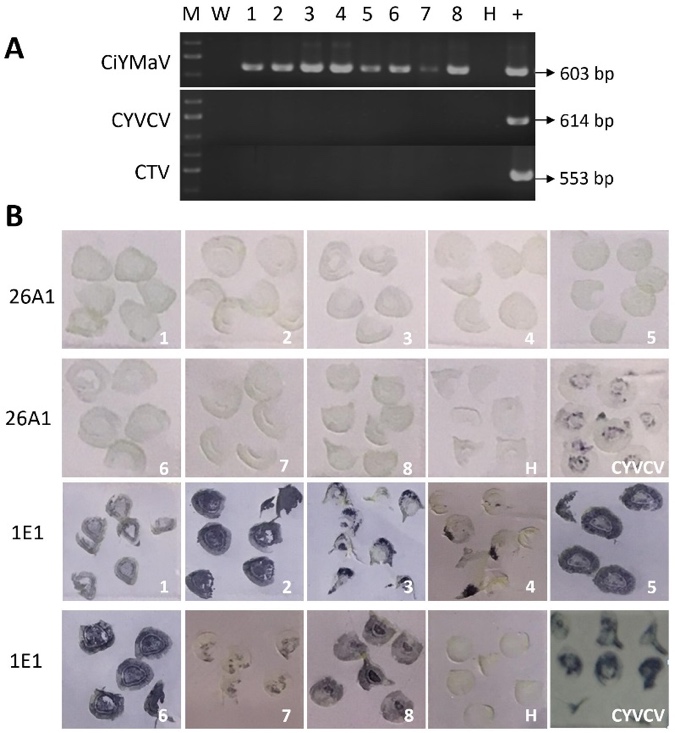




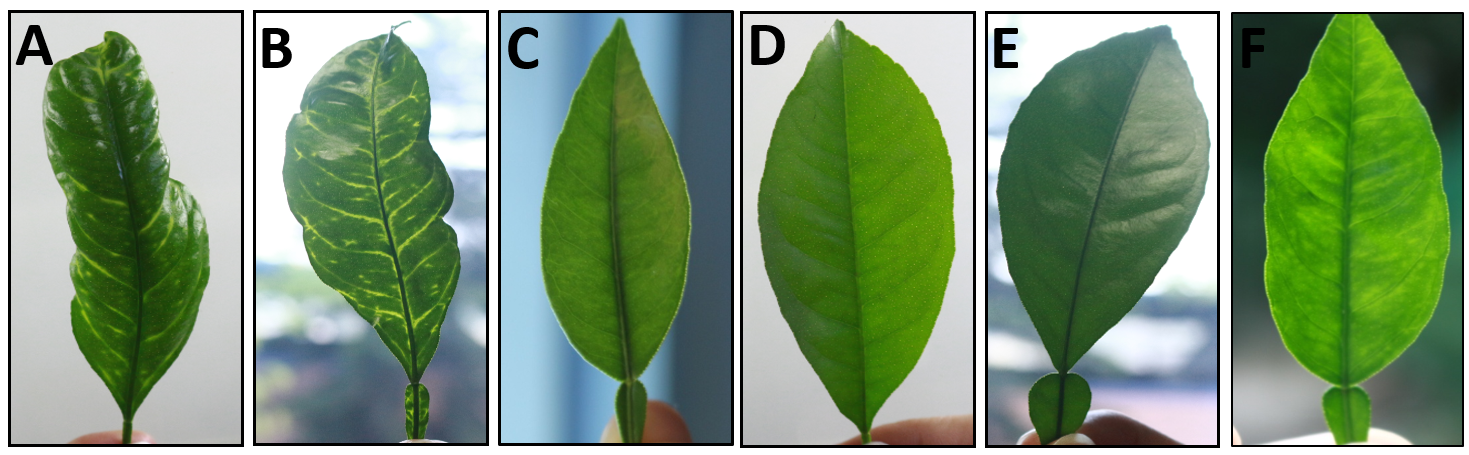
**C**



**Figure 3.** Transmission electron microscopy observations (after negative staining) of the extracts from fresh leaves of symptomatic citrus yellow mottle-associated virus-infected Symons sweet orange trees. The filamentous and flexuous viral-like particles are 600–700 nm long and 10–20 nm wide.



**Figure 4.** Detection of citrus yellow mottle-associated virus (CiYMaV), inoculated on different citrus varieties, using reverse transcription-polymerase chain reaction (RT-PCR) and direct tissue blot immunoassay (DTBIA) techniques. **(A)** RT-PCR analysis of CiYMaV, citrus yellow vein clearing virus (CYVCV), and citrus tristeza virus (CTV). M, DL2000 marker; W, water control; lanes 1–8, corresponds to samples of 1–8; lane H, healthy control; lane +, positive controls (PCV31 isolate in the case of CiYMaV; singly infected isolates from the Citrus Research Institute virus collection in the case of CTV and CYVCV). **(B)** Serological detection of samples from different citrus varieties infected CiYMaV or CYVCV by DTBIA using two monoclonal antibodies (1E1 and 26A1) specific for CYVCV. Samples 1 to 8 are infected by CiYMaV: 1, Dweet tangor; 2, Morocco sour orange; 3,6,7, Eureka lemon; 4, Daidai sour orange; 5, Chandler pummelo; 8, Symons sweet orange; H, non-infected control; CYVCV, CYVCV-infected SSO, positive control.



**Figure 5.** Symptoms observed in different citrus varieties infected with citrus yellow mottle-associated virus (CiYMaV) and citrus yellow vein clearing virus (CYVCV). **(A-B)** Vein clearing on Eureka lemon (EL) and Daidai sour orange (DSO) respectively, infected with CYVCV. (**C**) No symptom on Symons sweet orange (SSO) infected by CYVCV. **(D-E)** No symptom on EL and DSO respectively, infected by CiYMaV. (**F**) Yellowing and mottling on SSO infected by CiYMaV.

**Table 1.** Pairwisenucleotide and amino acid sequence identity between citrus yellow mottle-associated virus (CiYMaV) and sequence variants of citrus yellow vein clearing virus (CYVCV), Indian citrus ringspot virus (ICRSV) belonging to the genus *Mandarivirus*

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| **Isolate** | **Gen.** | **5’UTR** | | **ORF1 (REP)** | | | **ORF2 (TGB1)** | | | **ORF3 (TGB2)** | | | **ORF4 (TGB3)** | | **ORF5 (CP)** | | | **ORF6 (23K)** | | | **3’UTR** | |
| **nt%** | **nt%** | **nt%** | | **aa%** | **nt%** | | **aa%** | **nt%** | | **aa%** | **nt%** | | **aa%** | | **nt%** | **aa%** | | **nt%** | **aa%** | | **nt%** |
| CYVCV-Y1 (JX040635) | 72.08 | 82.05 | 72.89 | | 82.07 | 64.01 | | 63.72 | 71.21 | | 67.57 | 71.58 | | 72.13 | | 70.59 | 75.15 | | 76.83 | 70.40 | | 100 |
| CYVCV-PK (KP313241) | 72.30 | 82.05 | 73.31 | | 82.67 | 64.16 | | 63.72 | 70.61 | | 68.47 | 70.49 | | 70.49 | | 70.39 | 74.55 | | 76.98 | 70.85 | | 100 |
| CYVCV-YN (KP313242) | 72.43 | 82.05 | 73.31 | | 82.73 | 64.16 | | 63.72 | 70.91 | | 68.47 | 69.40 | | 68.85 | | 71.40 | 75.15 | | 76.98 | 71.30 | | 100 |
| ICRSV-K1 (NC\_003093) | 70.12 | 76.92 | 70.54 | | 80.89 | 61.06 | | 61.95 | 65.76 | | 65.77 | 73.22 | | 78.69 | | 72.90 | 74.85 | | 80.87 | 78.48 | | 97.22 |
| ICRSV-Pu (HQ324250) | 69.86 | 78.21 | 70.32 | | 80.35 | 60.91 | | 62.83 | 66.67 | | 67.57 | 71.58 | | 78.69 | | 72.20 | 73.64 | | 80.72 | 76.23 | | 97.22 |

**References**

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