

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

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| **Code assigned:** | **2022.012P** |  |
| **Short title:** Create two new species in the genus *Tymovirus* and one new species in the genus *Marafivirus* (*Tymovirales*:*Tymoviridae*)  |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Tymoviridae* Study Group, Plant Viruses Subcommittee |

**ICTV Study Group comments and response of proposer**

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**ICTV Study Group votes on proposal**

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| **Study Group** | **Number of members** |
| **Votes support** | **Votes against** | **No vote** |
| *Tymoviridae* | 4 |  | 2 |
|  |  |  |  |

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

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| **Is any taxon name used here derived from that of a living person (Y/N)** |  |

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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 | May 26, 2022 |
| 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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| 2022.012P.A.v2.Tymoviridae\_3nsp.xlxs |

**Abstract**

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| This proposal describes three novel viruses from naranjilla and sorghum that are related but distinct from current members of the family *Tymoviridae*. Accordingly, we propose creation of three new species, two in the genus *Tymovirus* and one in the genus *Marafivirus*, to classify these viruses. |

**Text of proposal**

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| Proposal to create 2 new species in the genus *Tymovirus* and 1 new species in the genus *Marafivirus* **A. Currently applied criteria for species demarcation in the genus *Tymovirus* (Dreher et al., 2012) re:*** Overall genome sequence identity of less than 80%.
* Capsid protein sequences less than 90% identical.
* Differential host range.
* Differences in the 3′-terminal structure.
* Serological specificity.

**1. Naranjilla chlorotic mosaic virus (NarCMV; *Tymovirus*; Green et al., 2018; MG323924)**Naranjilla chlorotic mosaic virus (NarCMV) was isolated and characterized by Green et al. (2018) from naranjilla (*Solanum quitoense* Lam.), a perennial shrub species cultivated in subtropical areas of Ecuador and Colombia. In 2015, a plant exhibiting symptoms of stunting, mosaic and chlorotic spots was sampled from the Pastaza province of Ecuador and spherical particles, composed of a single 20 kDa capsid protein, were observed under electron microscopy upon mechanical transmission to *Nicotiana benthamiana* indicator plants. High-throughput sequencing (HTS) was conducted from nucleic acids extracted from the indicator plant, and a single sequence contig was obtained that shared close relationships with tymoviruses. The complete 6,245 nucleotide sequence of the genome (MG323924) revealed a genome organization of three open reading frames (ORFs), a conserved tymobox typical of tymoviruses, a 3’ terminal 150 nt segment with a t-RNA-like secondary structure, and shared 71% sequence identity with the closest tymovirus, eggplant mosaic virus (EMV; KJ690172). This is below the 80% threshold for species demarcation in the genus *Tymovirus* in the family *Tymoviridae* (Dreher et al., 2012). The longest ORF encodes a 200 kDa protein containing replication-related domains, which shares 73% aa sequence identity with its closest relative, EMV. A smaller ORF which almost completely overlaps the 200 kDa protein, encodes a 69 kDa protein, and the 3’ proximal ORF, encodes the 188 aa coat protein (CP) of 20 kDa. The amino acid sequence of the CP was 83% identity to its closest match, chiltepin yellow mosaic virus (ChiYMV; FN563124), below the 90% threshold for species demarcation in the *Tymoviridae* Dreher et al., 2012). Phylogenetic analysis of the replicase and CP amino acids reveals that NarCMV is closely related, but distinct from EMV, ChiYMV and NarMMV (described below; Fig 1). Therefore, based on the overall nucleotide sequence identity of less than 80%, the identify of capsid protein sequences of less than 90%, and differential host range, NarCMV represents a distinct species in the genus *Tymovirus*, for which the name *Tymovirus naranjillae* is proposed. **2. Naranjilla mild mosaic virus (NarMMV; *Tymovirus*; Cornejo-Franco et al., 2019; MH784952)**In 2017, leaves from a Naranjilla (*Solanum quitoense* Lam.) plant exhibiting mosaic symptoms were collected from a commercial orchard in the Pinchincha Province of Ecuador (Cornejo-Franco et al., 2019). A host range studied revealed that the virus, designated naranjilla mild mosaic virus (NarMMV), systemically infected *Nicotiana benthamiana*, tamarillo, and eggplant upon mechanical inoculation. Total RNA extracted from greenhouse-inoculated tamarillo (*S. betaceum* Cav.) was used for HTS. The complete genome sequence of 6,348 nts (MH784952) was most closely related to ChiYMV (FN563124). Genome organization consisted of three ORFs (207 kDa replicase protein, 69 kDa, and the 3’ terminal CP ORF) a conserved 16 nt tymobox, and 3’ terminal tRNA-like secondary structure characteristic of the genus *Tymovirus* (Dreher et al., 2012). BLASTn analyses of the complete nucleotide sequence showed the highest identity between NarMMV and ChiYMV (74%) followed by naranjilla chlorotic mosaic virus (NarCMV; MG323924) at 70% and eggplant mosaic virus (EMV; KJ690172) at 68% (Cornejo-Franco et al., 2019). With the species demarcation criteria within the genus *Tymovirus* of less than 80% overall nucleotide sequence identity, the newly assembled genome places NarMMV in a distinct species within this genus (Dreher et al., 2012). At the amino acid level, identities observed for the 207 kDa replicase protein were 70% with NarCMV, and 68% with NarCMV and EMV. For the CP, the highest identity was found with ChiYMV (90%, followed by NarCMV of 80%, and EMV (76%). Phylogenetic relationships inferred from analysis of the replicase and CP amino acids indicate the NarMMV is most closely related to ChiYMV in a clade shared by NarCMV, EMV, and Andean potato mild mosaic virus (APMMV) (Fig 1). Based on the species demarcation criteria for new species in the genus *Tymovirus*, NarCMV is a closely related, but distinct, tymovirus from naranjilla plants; therefore, should be classified in a novel species for which we propose the name *Tymovirus quitoense*. (Supporting evidence is provided in maximum likelihood phylogenetic trees generated for the replicase and coat protein amino acid sequences in Figure 1). **B. Currently applied criteria for species demarcation in the genus *Marafivirus* are (Dreher et al., 2012)*** Overall genome sequence identity of less than 80%.
* Capsid protein sequences less than 90% identical.
* Differential host range.
* Differences in the 3′-terminal structure and the number of ORFs
* Vector specificity
* Serological specificity.

**1. Sorghum almum marafivirus (SoAMV); *Marafivirus*; Boukari et al., 2021; MN714643)**In 2021, a tentative new marafivirus species was identified in *Sorghum almum* and *Sorghum bicolor* in Florida, and collected in 2015 and 2016 (Boukari et al., 2021). RNA extracted from leaf samples was subjected to HTS revealed the presence of two sorghum marafiviruses, one from *S. almum* (SoAMV; MN714643) and one from *S. bicolor* (SoBMV; MN100128). The marafivirus consensus sequence from *S. almum* was 6,138 nt and BLASTn analysis revealed 76% nt sequence identity to maize rayado fino virus (MRFV; HM133581) in the genus *Marafivirus*, family *Tymoviridae*. The marafivirus sequences from *S. bicolor* and *S. almum* shared 82% nt sequence identity over 96% of their genome. Based on phylogenetic analyses of the aa sequence of the CP, the two virus isolates from sorghum in Florida formed a unique lineage distinct from other marafiviruses (Fig 1). Based on the species demarcation criteria, these viruses represent two isolates of a new marafivirus species for which we propose the name *Marafivirus sorghi*. (Supporting evidence is provided in maximum likelihood phylogenetic trees generated for the replicase and coat protein amino acid sequences in Figure 1).  |

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**Supporting evidence**

**Figure 1. Phylogenetic trees**

**Figure 1. A. Evolutionary analysis of CP amino acids using the Maximum Likelihood method**
The evolutionary history was inferred by using the Maximum Likelihood method and General Reverse Transcriptase + Freq. model. The tree with the highest log likelihood (-13599.10) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree includes unassigned viruses. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1.7632)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 51 amino acid sequences. There were a total of 261 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. The GenBank Accession numbers for the CP are: Andean potato latent virus (APLV) - JX508291.1; Andean potato mild mosaic virus (APMM) - JX508290.1; Asclepias asymptomatic virus (AsAV) - NC\_015523.1; alfalfa virus F (AVF) - NC\_040565.1; Anagyris vein yellowing virus (AVYV) - AY751780.1; Bermuda grass etched-line virus (BELV) - AY040531.1; belladonna mottle virus (BeMV) - X54529.1; Bombyx mori latent virus (BmLV) - AB186123.1; blackberry virus S (BVS) - FJ915122.1; Calopogonium yellow vein virus (CalYVV) - U91413.1; chiltepin yellow mosaic virus (ChiYMV) - FN563123.1; chayote mosaic virus (ChMV) - AF195000.2; citrus sudden death-associated virus (CSDaV) - AY884005.1; cacao yellow mosaic virus (CYMV) - X54354.1; Clitoria yellow vein virus (CYVV) - AF035200.1; Diascia yellow mottle virus (DiaYMV) - NC\_011086.1; Dulcamara mottle virus (DuMV) - AY789137.1; Desmodium yellow mottle virus (DYMoV) - AF035201.1; eggplant mosaic virus (EMV) - J04374.1; Erysimum latent virus (ErLV) - AF098523.1; grapevine asteroid mosaic associated virus (GAMaV) - NC\_031692.1; grapevine-associated marafivirus (GaMV) - MZ422607.1; grapevine fleck virus (GFkV) - AJ309022.1; grapevine Red Globe virus (GRGV) - KX171166.1; grapevine rupestris vein feathering virus (GRVFV) - NC\_034205.1; grapevine Syrah virus 1 (GSyV-1) - FJ436028.1; grapevine virus Q (GVQ) - FJ977041.1; kennedya yellow mosaic virus (KYMV) - D00637.1;maize rayado fino virus (MRFV) - AF265566.1; Medicago sativa marafivirus 1 (MsMV1) - MF443260.1; naranjilla chlorotic mosaic virus (NarCMV) - MG323924.1; naranjilla mild mosaic virus (NarMMV) - MH784952.1; Nemesia ring necrosis virus (NeRNV) - AY751778.1; nectarine marafivirus M (NeVM) - KT273411.1; oat blue dwarf virus (OBDV) - U87832.1; okra mosaic virus (OkMV) - EF554577.1; olive latent virus 3 (OLV-3) - FJ444852.2; ononis yellow mosaic virus (OYMV) - J04375.1; petunia vein banding virus (PetVBV) - AF210709.2; passion fruit yellow mosaic virus (PFYMV) - KY823429.1; Physalis mottle virus (PhyMV) - Y16104.1; Plantago mottle virus (PlMoV) - AY751779.1; poinsettia mosaic virus (PnMV) - AJ271595.1; peach virus D (PVD) - NC\_033828.1; Sorghum almum marafivirus (SoAMV) - MN714643.1; Sorghum bicolor marafivirus (SoBMV) - MN100128.1; Scrophularia mottle virus (SrMV) - AY751777.1; switchgrass mosaic virus (SwMV) - NC\_015522.1; tomato blistering mosaic virus (ToBMV) - KC840043.1; turnip yellow mosaic virus (TYMV) - X07441.1; wild cucumber mosaic virus (WCMV) - AF035633.1.



Figure 1A Coat Protein

**B. Evolutionary analysis of replicase amino acids using the Maximum Likelihood method**
The evolutionary history was inferred by using the Maximum Likelihood method and General Reverse Transcriptase + Freq. model. The replicase included the amino acids between the methyl transferase domains to the RdRp domain + 60 amino acids downstream. The tree includes unassigned viruses. The range of the replicase amino acid length is 1,630 – 1,837. The tree with the highest log likelihood (-95495.69) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.8365)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 6.60% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 43 amino acid sequences. There were a total of 2307 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. The GenBank Accession numbers for the replicase are: Andean potato latent virus (APLV) - JX508291.1; Andean potato mild mosaic virus (APMM) - JX508290.1; Asclepias asymptomatic virus (AsAV) - NC\_015523.1; alfalfa virus F (AVF) - NC\_040565.1; Anagyris vein yellowing virus (AVYV) - AY751780.1; Bombyx mori latent virus (BmLV) - AB186123.1; blackberry virus S (BVS) - FJ915122.1; chiltepin yellow mosaic virus (ChiYMV) - FN563123.1; chayote mosaic virus (ChMV) - AF195000.2; citrus sudden death-associated virus (CSDaV) - AY884005.1; Diascia yellow mottle virus (DiaYMV) - NC\_011086.1; Dulcamara mottle virus (DuMV) - AY789137.1; eggplant mosaic virus (EMV) - J04374.1; Erysimum latent virus (ErLV) - AF098523.1; grapevine asteroid mosaic associated virus (GAMaV) - NC\_031692.1; grapevine-associated marafivirus (GaMV) - MZ422607.1; grapevine fleck virus (GFkV) - AJ309022.1; grapevine Red Globe virus (GRGV) - KX171166.1; grapevine rupestris vein feathering virus (GRVFV) - NC\_034205.1; grapevine Syrah virus 1 (GSyV-1) - FJ436028.1; grapevine virus Q (GVQ) - FJ977041.1; Kennedya yellow mosaic virus (KYMV) - D00637.1; maize rayado fino virus (MRFV) - AF265566.1; Medicago sativa marafivirus 1 (MsMV1) - MF443260.1; naranjilla chlorotic mosaic virus (NarCMV) - MG323924.1; naranjilla mild mosaic virus (NarMMV) - MH784952.1; Nemesia ring necrosis virus (NeRNV) - AY751778.1; nectarine marafivirus M (NeVM) - KT273411.1; oat blue dwarf virus (OBDV) - U87832.1; okra mosaic virus (OkMV) - EF554577.1; olive latent virus 3 (OLV-3) - FJ444852.2; Ononis yellow mosaic virus (OYMV) - J04375.1; passion fruit yellow mosaic virus (PFYMV) - KY823429.1; Physalis mottle virus (PhyMV) - Y16104.1; Plantago mottle virus (PlMoV) - AY751779.1; poinsettia mosaic virus (PnMV) - AJ271595.1; peach virus D (PVD) - NC\_033828.1; Sorghum almum marafivirus (SoAMV) - MN714643.1; Sorghum bicolor marafivirus (SoBMV) - MN100128.1; Scrophularia mottle virus (SrMV) - AY751777.1; switchgrass mosaic virus (SwMV) - NC\_015522.1; tomato blistering mosaic virus (ToBMV) - KC840043.1; turnip yellow mosaic virus (TYMV) - X07441.1.



Figure 1B Replicase

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