This form should be used for all taxonomic proposals. Please complete all those modules that are applicable.

For guidance, see the notes written in blue and the separate document “Help with completing a taxonomic proposal”

Please try to keep related proposals within a single document.

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

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| **Code assigned:** | ***2017.019P*** | | | | (to be completed by ICTV officers) |
| **Short title:** Two new species in the genus *Marafivirus* (family *Tymoviridae*) | | | | | |
| **Modules attached**  (Modules 1, 4 and either 2 or 3 are required. | | **1** **X 2 X 3  4 X** | | | |
| **Author(s):** | | | | | |
| Sead Sabanadzovic  Mike Edwards  Rose Hammond  Anne-Lise Haenni  Giovanni P Martelli  Theo Dreher | | | | | |
| **Corresponding author with e-mail address:** | | | | | |
| **Sead Sabanadzovic** [ss501@msstate.edu](mailto:ss501@msstate.edu) | | | | | |
| **List the ICTV study group(s) that have seen this proposal:** | | | | | |
| A list of study groups and contacts is provided at <http://www.ictvonline.org/subcommittees.asp> . If in doubt, contact the appropriate subcommittee chair (there are six virus subcommittees: animal DNA and retroviruses, animal ssRNA-, animal ssRNA+, fungal and protist, plant, bacterial and archaeal) | | | ICTV *Tymoviridae* SG | | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | | | |
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| Date first submitted to ICTV: | | | | 2017 | |
| Date of this revision (if different to above): | | | |  | |

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| **ICTV-EC comments and response of the proposer:** |
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**Part 2**: **PROPOSED TAXONOMY**

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| Present the proposed new taxonomy on accompanying spreadsheet |
| **Name of accompanying spreadsheet: 2017.019P.N.v1.Marafivirus\_2sp** |

Please display the taxonomic changes you are proposing on the accompanying spreadsheet module 2017\_TP\_Template\_Excel\_module. Submit both this and the spreadsheet to the appropriate ICTV Subcommittee Chair.

**Part 4:** **APPENDIX**: supporting material

| additional material in support of this proposal |
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| **References:** |
| **Abou Ghanem-Sabanadzovic N., S. Sabanadzovic, G.P. Martelli, 2003**. Sequencing of the 3' end of three grapevine fleck virus-like viruses. Virus Genes 27:11-16.  **Boscia D., S. Sabanadzovic, V. Savino, P.E. Kyriakopoulou, G.P. Martelli, 1994.** A non mechanically transmissible virus associated with asteroid mosaic of the grapevine. Vitis 33:101-102.  **Castresana J., 2000.** Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17: 540-552.  **Chevenet F., C. Brun, A.L. Banuls, B. Jacq, R. Chisten, 2006**. TreeDyn: towards dynamic graphics and annotations for analyses of trees. BMC Bioinformatics 10:439.  **Dereeper A., V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J.F. Dufayard, S. Guindon, V. Lefort, M. Lescot, J.M. Claverie, O. Gascuel, 2008.** Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36(Web Server issue):W465-9.  **Dreher T.W., M.C. Edwards, A-L. Haenni, R.W. Hammond, I. Jupin, R. Koenig, S. Sabanadzovic, and G.P. Martelli, 2012**. Family *Tymoviridae*. In: A.M.Q. King, E. Lefkowitz, M.J. Adams, E.B. Carstens (Eds.) Virus Taxonomy - Ninth Report of the ICTV. Elsevier Academic Press, San Diego, 913-921.  **Edgar R.C., 2004.** MUSCLE: multiple sequence alignment with high accuracy and high throughput, Nucleic Acids Res 32: 1792-1797.  **Hewitt W.B., 1954.** Some virus and virus-like diseases of grapevines. Bull California Dept Agric 43:47-64.  **Huelsenbeck J.P., F. Ronquist, 2001**. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755  **Igori D., S. Lim, D. Baek, S.Y. Kim, E. Seo, I-S. Cho, G-S. Choi, H-S. Lim, J.S. Moon, 2017.** Complete nucleotide sequence and genome organization of peach virus D, a putative new member of the genus *Marafivirus*. Arch Virol 162:1769-1772. Vargas-Asencio J., K. Wojciechowska, M. Baskerville, A.L. Gomez, K.L. Perry, J.R. Thompson, 2017. The complete nucleotide sequence and genomic characterization of grapevine asteroid mosaic associated virus. Virus Res 227:82-87. |

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| **Annex:**  Please explain the reasons for the taxonomic changes you are proposing and provide evidence to support them. The following information should be provided, where relevant:   * **Species demarcation criteria**: Explain how new species differ from others in the genus and demonstrate that these differences meet the criteria previously established for demarcating between species. If no criteriahave previously been established, and if there will now be more than one species in the genus, please state the demarcation criteria you are proposing. * **Higher taxa**:   + There is no formal requirement to state demarcation criteria when proposing new genera or other higher taxa. However, a similar concept should apply in pursuit of a rational and consistent virus taxonomy.   + Please indicate the **origin of names** assigned to new taxa at genus level and above.   + For each new genus a **type species** must be designated to represent it. Please explain your choice. * **Supporting evidence**: The use of Figures and Tables is strongly recommended (note that copying from publications will require permission from the copyright holder). For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance. |

**Proposal to create two new species in the genus *Marafivirus,* family *Tymoviridae***

Currently applied criteria for species demarcation in the genus *Marafivirus* (family *Tymoviridae*) are (Dreher et al., 2012):

* Overall sequence identity less than 80%
* Coat protein sequences less than 90% identical
* Differences in the 3' terminal structure
* Differential host range
* Serological specificity

In this taxoprop we propose creation of two new species in the genus *Marafivirus* in order to classify two recently described viruses with properties resembling members of that taxon.

The genome of both viruses possess the essential properties of marafiviruses (single ORF encompassing replication and coat proteins; marafibox, poly(A) tail) and are well differentiated by sequence identity and host range from recognized members of the genus *Marafivirus*. Detailed justification is presented below based upon recently published data (Igori et al., 2017; Vargas-Asencio et al., 2017).

**Grapevine asteroid mosaic associated virus (GAMaV)**

GAMaV is an isometric and phloem-limited virus of ca 30 nm in diameter, originally reported from grapevines affected by "asteroid mosaic" (Boscia et al., 1994), a disease originally reported from California (Hewitt, 1954). Until recently, the genome of this virus was only partially sequenced (Abou Ghanem-Sabanadzovic et al., 2003), which impeded its classification. In 2017, the genome of the GAMaV isolate GV30 has been completely sequenced by combination of high throughput (Illumina) and Sanger sequencing methodologies and viral termini were obtained with RACE (Vargas-Asencio et al., 2017).

The complete genome of GAMaV-GV30 is 6,719 nt long excluding a poly(A) tail. The 5' and 3' untranslated regions are 129 and 116 nt long, respectively. The monocistronic genome encodes a putative polyprotein of 2158 amino acids characterized by the presence of conserved domains of a methyltransferase (MTR), protease/endopeptidase (PRO), a viral helicase (HEL), an RNA dependent RNA polymerase (RdRp), and coat proteins (CPs) (Fig. 1).

Direct comparison with previously available 3'-proximal 1,852 nt-long sequence (GenBank AJ249357; Abou Ghanem-Sabanadzovic et al., 2003) revealed identity of 94% between the two isolates. GAMaV-GV30 shared nt identities of 72% (78% coverage) with citrus sudden death associated virus (CSDaV; GenBank AY884005.1), 71% (76% coverage) with nectarine virus M (NeVM; KT273411) and 74% (75% coverage) with oat blue dwarf virus (OBDV;GU396990.1). A conserved maraﬁbox-like sequence was identified at nt position 5,875–5,891. The major CP is predicted to be 21.1 kDa and 198 aa long, while the minor CP is estimated to have a molecular mass of 22.9 kDa (216 aa). BLASTp analysis of the major CP shows the highest identity of 99% with the USA9 isolate of GAMaV (CAC10493) followed by CSDaV and NeVM (both 70%) and 68% with OBDV (Vargas-Asencio et al., 2017).

Based upon the above presented results, and phylogenetic analyses (Fig. 2),GAMaV-GV30 is a representative of a new species in the genus *Marafivirus* for which the name *Grapevine asteroid mosaic associated marafivirus* is proposed.

**Peach virus D (PeVD)**

The complete nucleotide sequence of a new virus isolated from leaves of *Prunus persica* showing yellowing and mottling, collected in South Korea, was determined with Illumina and Sanger sequencing (Igori et al., 2017). The genome of this virus (GenBank KY084481), provisionally named peach virus D (PeVD) consists of 6,612 nucleotides excluding the 3' poly(A) tail and contains a single open reading frame potentially coding for a large precursor polyprotein of 227 kDa containing conserved domains of viral methyltransferase (MTR), protease/endopeptidase (PRO), viral RNA helicase superfamily 1 (HEL), RNA-dependent RNA polymerase (RdRp) and coat protein (CP) (Fig. 1). Sequence comparisons and phylogenetic analysis revealed that PeVD is most closely related to viruses in the genus *Marafivirus*, family *Tymoviridae*. The complete nucleotide and CP amino acid sequences of PeVD were most similar (51.1–57.8% and 32.2–48.0%, respectively) to members of the genus *Marafivirus*. These results, along with results of phylogenetic affiliation with recognized marafiviruses (Fig. 2), suggest that PeVD should be regarded as a member of a distinct species in the genus *Marafivirus* for which the name *Peach marafivirus D* is proposed.

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**Figure 1.** Diagrammatic representation of the genome organization of GAMaV and PeVD. The box represents the single ORF and lines represent untranslated regions (UTRs). Abbreviations: MTR = methyltransferase, PRO = endopeptidase/protease, Hel = helicase, RdRp = RNA-dependent RNA polymerase, CP = coat protein, box = "marafibox".

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**Figure 2.** Bayesian phylogenetictree showing relationships of GAMaV and PeVD (reported in red) with maraﬁviruses and selected members of the family *Tymoviridae* based on the amino acid sequences of viral RNA-dependent RNA polymerase. The analysis was performed on the Phylogeny.fr online resource (Dereeper et al., 2008) including initial alignment with Muscle (Edgar, 2004) and curation with Gblocks (Castresana, 2000). The phylogenetic tree was reconstructed with MrBayes v3.2.3 (Huelsenbeck and Ronquist, 2001) under the default settings and visualized with TreeDyn (Chevenet et al., 2006). Percentage bootstrap support is reported on each node. Acronyms, names and corresponding GenBank accession numbers of viruses used in the analysis are: AVYV (Anagyris vein yellowing virus, AY751780.1), BVS (blackberry virus S, FJ915122), CSDaV (citrus sudden death-associated virus, DQ185573), DuMV (dulcamara mottle virus, AY789137.1), EMV (eggplant mosaic virus, J04374.1), GAMaV (grapevine asteroid mosaic associated virus, KX354202), GFkV (grapevine ﬂeck virus, AJ309022), GRVFV (grapevine rupestris vein feathering virus, AY706994), GSyV1 (grapevine Syrah virus 1, JX513896), KYMV (Kennedya mosaic virus, D00637), MRFV (maize rayado fino virus, KM523134), NeVM (nectarine virus M, KT273411), OBDV (oat blue dwarf virus, GU396990), OkMV (okra mosaic virus, EF554577.1), OLV3 (olive latent virus 3, FJ444852.2), OYMV (Ononis yellow mosaic virus, J04375.1), PeVD (peach virus D, KY084481), PhyMV (physallis mottle virus, Y16104.1), PnMV (poinsettia mosaic virus, AM412237), SrMV (Scrophularia mottle virus, AY751777.1), SwMV (switchgrass mosaic virus, JF727261.2), TYMV (turnip yellow mosaic virus, X07441).