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**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Code assigned:** | ***2017.020P*** | | | | (to be completed by ICTV officers) |
| **Short title:** 3 new species in a proposed new genus *Deltaflexivirus* and new family *Deltaflexiviridae* in the order *Tymovirales* | | | | | |
| **Modules attached**  (Modules 1, 4 and either 2 or 3 are required. | | **1**  **2  3  4** | | | |
| **Author(s):** | | | | | |
| Jan Kreuze (chair) [j.kreuze@cgiar.org](mailto:j.kreuze@cgiar.org)  Adams, Michael J. [mike.adams.ictv@gmail.com](mailto:mike.adams.ictv@gmail.com)  Candresse, Thierry [tc@bordeaux.inra.fr](mailto:tc@bordeaux.inra.fr)  Hammond, John [John.Hammond@ARS.USDA.GOV](mailto:John.Hammond@ARS.USDA.GOV)  Menzel, Wulf [Wulf.Menzel@dsmz.de](mailto:Wulf.Menzel@dsmz.de)  Pearson, Michael [m.pearson@auckland.ac.nz](mailto:m.pearson@auckland.ac.nz)  Saldarelli, Pasquale [p.saldarelli@ba.ivv.cnr.it](mailto:p.saldarelli@ba.ivv.cnr.it)  Vaira, Anna Maria [a.vaira@ivv.cnr.it](mailto:a.vaira@ivv.cnr.it)  Yoshikawa, Nobuyuki [yoshikawa@iwate-u.ac.jp](mailto:yoshikawa@iwate-u.ac.jp) | | | | | |
| **Corresponding author with e-mail address:** | | | | | |
| Jan Kreuze [j.kreuze@cgiar.org](mailto:j.kreuze@cgiar.org) | | | | | |
| **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) | | | **Flexiviruses** | | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | | | |
|  | | | | | |
|  | | | | | |
| Date first submitted to ICTV: | | | | June 2017 | |
| Date of this revision (if different to above): | | | |  | |

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

**Part 2**: **PROPOSED TAXONOMY**

|  |
| --- |
| Present the proposed new taxonomy on accompanying spreadsheet |
| **Name of accompanying spreadsheet: 2017.020P.N.v1.Deltaflexiviridae** |

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 |
| --- |
| **References:** |
| Chen X., He, H., Yang, X., Zeng, H., Qiu, D & Guo, L. (2016). The complete sequence of a novel *Fusarium graminearum* RNA virus in a new proposed family within the order *Tymovirales*. *Archives of Virology* 161:2899-2903. doi 10.1007/s00705-016-2961-1  Li, K., Zheng, D., Cheng, J., Chen, T., Fu, Y., Jiang, D., & Xie, J. (2016). Characterization of a novel *Sclerotinia sclerotiorum* RNA virus as the prototype of a new proposed family within the order *Tymovirales*. *Virus Research* 219: 92-99. doi: 10.1016/j.virusres.2015.11.019  Marzano, S-Y.L. & Domier, L.L. (2016). Novel mycoviruses discovered from metatranscriptomics survey of soybean phyllosphere phytobiomes. *Virus Research* 213: 332-342. Doi: 10.1016/j.virusres.2015.11.002 |

**Annex:**

Three viruses recently reported (probably all mycoviruses) are clearly related to one another and it has been suggested (Li et al., 2016; Chen et al., 2016) that they justify the creation of a new family, *Deltaflexiviridae*, within the order *Tymovirales*. Details of the viruses are as follows:

**Sclerotinia sclerotiorum deltaflexivirus 1 (****SsDFV1) (Li et al., 2016; KT581451)**

Double-stranded RNA was extracted from an isolate of the plant pathogen, *Sclerotinia sclerotiorum* and a band of ~8 kb was identified in PAGE. After purification, this was used as a template for RT-PCR using random primers and further RT-PCR and RACE steps were used to amplify and then assemble a virus sequence of 8148 nt excluding the polyA tail. A large ORF (coding for a polyprotein of 2075 aa; 230kDa) was identified and three smaller ORFs downstream (Figure 1) that had no recognizable similarity to other sequences in Genbank. The large polyprotein had methyltransferase, helicase and RNA-dependent RNA polymerase (RdRp) domains with distant similarity to members of the order *Tymovirales* (generally 25-30% aa identity within these conserved domains). Attempts to isolate virions from the fungal mycelium were unsuccessful and the authors conclude that the virus is probably not encapsidated. Phylogenetic trees presented for the methyltransferase and helicase domains by the authors appear to show SsDFV1 as separate branch within the order *Tymovirales* although these branches lack bootstrap support. A tree for the RdRp domain or the complete replication protein (rooted with a member of the family *Hypoviridae*) show SsDFV1 slightly below the root of the tree for the current order but the authors nevertheless propose that it represents a new species, new genus and new family in the order.

**Soybean leaf-associated mycoflexivirus 1 (****SlaMFV1) (Marzano & Domier, 2016;** **KT598226)**

In a metatranscriptomics study of soybean leaf tissue, a large virus contig (8327 nt; KT598226) was consistently assembled. The sequence contained one large open reading frame and three small downstream ORFs were also predicted (Figure 1). The predicted amino acid sequence of ORF1 product (2084 aa; 232 kDa) contained methyltransferase, helicase and RNA-dependent RNA polymerase (RdRp) domains and was most similar (‘a maximum of 43% aa identity’) to the replicase of an isolate of *Botrytis virus F*, the type member of the genus *Mycoflexivirus* (family *Gammaflexiviridae*, order *Tymovirales*) and the authors suggested that the sequence represents a genome of a new mycovirus. The predicted amino acid sequences of the downstream ORFs products did not show homology to other sequences in GenBank and a CP-encoding ORF could not be identified. The authors suggested that the sequence is almost complete because of its size in relation to established members of the order *Tymovirales*, but this has not been verified. Phylogenetic analysis presented by the authors shows the replication polyprotein as a distinct branch between members of the *Gammaflexiviridae* and *Tymoviridae* but with low (53%) bootstrap support.

**Fusarium graminearum deltaflexivirus 1 (****FgDFV1) (Chen et al., 2016;** **KX015962)**

Double-stranded RNA was extracted from an isolate of the plant pathogen, *Fusarium graminearum* and a band of ~8 kb was identified in PAGE. After purification, this was used as a template for RT-PCR using random primers and further RT-PCR and RACE steps were used to amplify and then assemble a virus sequence of 8246 nt excluding the polyA tail. Five putative ORFs were identified (Figure 1), the largest of which (ORF1: nt 36-6164) encoded a putative alpha-like replication polyprotein (2042 aa; 227 kDa) which had 48% aa identity to that predicted for SsDFV1. ORF2 had 41% identity to the predicted ORF3 of SlaMFV1 but there were no other similarities between the sequences of the smaller ORFs and other sequences in GenBank. A phylogenetic tree presented by the authors (rooted with a member of the family *Hypoviridae*) shows FgDFV1 grouping with SsDFV1 and SlaMFV1 but not with isolates of any other currently accepted species within the order *Tymovirales*.

**Evaluation**

These three viruses have replication polyproteins of the alpha supergroup that are clearly related to one another. SsDFV1 and SlaMFV1 have 69% identical amino acids and other comparisons (SsDFV1 or SlaMFV1 with FgDFV1) are in the region of 47% identity. It is not known if the other predicted ORFs are expressed and they have no homologies to known proteins but there are similarities between ORF2 of FgDFV1 and ORF3 of SlaMFV1. These similarities are reflected in robust clustering in phylogenetic analyses (Figure 2) that suggest that these viruses represent three distinct species. Without further evidence of the significance of the smaller ORFs, it seems best to allocate these to a single new genus and a new family is justified by their distance from any other known viruses.

The relationship with established members of the order *Tymovirales* is distant but there is good bootstrap support for a distant grouping with the sole current member of the family *Gammaflexiviridae* (Figure 2). This proposal therefore accepts the case for a further family in the order, with the name *Deltaflexiviridae* and consisting of a single genus.

***Species names:*** The names proposed reflect the virus names already published to maintain continuity with the literature, while recognizing that there is little evidence that these have the type of virions characteristic of the plant-infecting flexiviruses. However, there are some simplifications:

|  |  |  |
| --- | --- | --- |
| **Published virus name** | **Species name proposed** | **Note** |
| Sclerotinia sclerotiorum deltaflexivirus 1 | *Sclerotinia deltaflexivirus 1* | 1 |
| soybean leaf-associated mycoflexivirus 1 | *Soybean-associated deltaflexivirus 1* | 2 |
| Fusarium graminearum deltaflexivirus 1 | *Fusarium deltaflexivirus 1* | 1 |

Note1: simplified to host genus name

Note 2: simplified and modified to reflect new genus name

**3 new species proposed:** *Sclerotinia deltaflexivirus 1,* *Soybean-associated deltaflexivirus 1,* *Fusarium deltaflexivirus 1*

**1 new genus proposed:** *Deltaflexivirus*

**Species demarcation criteria**: differences in host range; differences in the number of minor ORFs; amino acid identities between the respective replication polyproteins of less than 70%.

Type species *Sclerotinia deltaflexivirus 1* (the first and best-described member).

**1 new family proposed:** *Deltaflexiviridae* **Order:** *Tymovirales*



**Figure 1.** Genome organisation of the three viruses described in this proposal. The large ORF in each virus is a replication polyprotein with methyltransferase (Mtr), helicase (Hel) and RNA-dependent RNA polymerase (RdRp) domains. The smaller ORFs downstream are predicted but the function of their products is unknown and it is not known if they are expressed although there are similarities between the two ORFs shown in green.



**Figure 2.** Neighbour-joining phylogenetic analysis of the entire replication protein amino acid sequences of representative members of the order *Tymovirales* and the three members of the proposed new family *Deltaflexiviridae* (in red). Tree prepared in MEGA7 with JTT amino acid substitutions and rooted with rubella virus (RUBV, genus *Rubivirus*, family *Togaviridae*; M15240). Bootstrap percentages from 1000 replicates are shown at the branches (where >60%). Other abbreviations and sequence accession numbers as follows: ACLSV, apple chlorotic leaf spot virus (genus *Trichovirus*; D14996); ASGV, apple stem grooving virus (genus *Capillovirus*; D14995); ASPV, apple stem pitting virus (genus *Foveavirus*; D21829); AVCaV, apricot vein clearing associated virus (genus *Prunevirus*; HG008921); BanMMV, banana mild mosaic virus (unassigned; AF314662); BmMLV, Bombyx mori latent virus (unassigned; AB186123); BotV-F, Botrytis virus F (genus *Mycoflexivirus*; AF238884); BotV-X, Botrytis virus X (genus *Botrexvirus*; AY055762); BVE, blackberry virus E (unassigned; JN053266); CChV-1, carrot Ch virus 1 (genus *Chordovirus*; KF533711); CLBV, Citrus leaf blotch virus (genus *Citrivirus*; AJ318061); CNRMV, cherry necrotic rusty mottle virus (genus *Robigovirus*; AF237816); DiV-A, Diuris virus A (genus *Divavirus*; JX173276); DOSV, donkey orchid symptomless virus (genus *Platypuvirus*; KC923234); FgDFV1, Fusarium graminearum deltaflexivirus 1 (KX015962); FgMV-1, Fusarium graminearum mycotymovirus 1 (unclassified; KT360947); GFkV, grapevine fleck virus (genus *Maculavirus*; AJ309022); GVA, grapevine virus A (genus *Vitivirus*; X75433); ICRSV, Indian citrus ringspot virus (genus *Mandarivirus*; AF406744); LoLV, Lolium latent virus (genus *Lolavirus*; EU489641); MRFV, maize rayado fino virus (genus *Marafivirus*; AF265566); PnMV, Poinsettia mosaic virus (unassigned; AJ271595); PVM, potato virus M (genus *Carlavirus*; D14449); PVT, potato virus T (genus *Tepovirus*; EU835937); PVX, potato virus X (genus *Potexvirus*; D00344); SlaMFV1, soybean leaf-associated mycoflexivirus 1 (KT598226); SCSMaV, sugarcane striate mosaic-associated virus (unassigned; AF315308); ShVX, shallot virus X (genus *Allexivirus*; M97264); SSDaV, Sclerotinia sclerotiorum debilitation-associated RNA virus (genus *Sclerodarnavirus*; AY147260); SsDFV1, Sclerotinia sclerotiorum deltaflexivirus 1 (KT581451); TYMV, turnip yellow mosaic virus (genus *Tymovirus*; X07441).