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:** | ***2017.021P*** | | | | (to be completed by ICTV officers) |
| **Short title:** 14 new species in the Alphaflexiviridae and Betaflexiviridae families and reassignment of existing species *Blackberry virus E* to the genus *Allexivirus* | | | | | |
| **Modules attached**  (Modules 1, 4 and either 2 or 3 are required. | | **1**  **2  3  4** | | | |
| **Author(s):** | | | | | |
| Jan Kreuze (chair)  Adams, Michael J.  Candresse, Thierry  Hammond, John  Menzel, Wulf  Pearson, Michael  Saldarelli, Pasquale  Vaira, Anna Maria  Yoshikawa, Nobuyuki | | [j.kreuze@cgiar.org](mailto:j.kreuze@cgiar.org)  [mike.adams.ictv@gmail.com](mailto:mike.adams.ictv@gmail.com)  [tc@bordeaux.inra.fr](mailto:tc@bordeaux.inra.fr)  [John.Hammond@ARS.USDA.GOV](mailto:John.Hammond@ARS.USDA.GOV)  [Wulf.Menzel@dsmz.de](mailto:Wulf.Menzel@dsmz.de)  [m.pearson@auckland.ac.nz](mailto:m.pearson@auckland.ac.nz)  [p.saldarelli@ba.ivv.cnr.it](mailto:p.saldarelli@ba.ivv.cnr.it)  [a.vaira@ivv.cnr.it](mailto:a.vaira@ivv.cnr.it)  [yoshikawa@iwate-u.ac.jp](mailto:yoshikawa@iwate-u.ac.jp) | | | |
| **Corresponding author with e-mail address:** | | | | | |
| [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) | | |  | | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | | | |
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|  | | | | | |
| Date first submitted to ICTV: | | | |  | |
| Date of this revision (if different to above): | | | |  | |

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

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

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| Present the proposed new taxonomy on accompanying spreadsheet |
| **Name of accompanying spreadsheet:** **2017.021P.N.v2.Alpha-Betaflexiviridae\_14sp** |

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 3:** **NON-STANDARD**

Template for any proposal regarding ICTV procedures, rules or policy, not involving the creation of new taxonomy.

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| non-standard proposal |
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| **Text of proposal:** |
|  |

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

| additional material in support of this proposal |
| --- |
| **References:** |
| * Ellis, P.J., Stace-Smith, R., Converse, R.H. (1992) Isolation and some properties of a North American carlavirus in *Sambucus racemosa*. Acta Hortic 308:113-120. * Gutierrez-Sanchez, P.A., Jaramillo-Mesa, H., Marin-Montoya, M.A. (2016) Next generation sequence analysis of the forage peanut (*Arachis pintoi*) virome. Rev Fac Nac Agron Medellin 69: 7881-7891 * Grisoni, M., Marais, A. *et al.* (2017) Two novel *Alphaflexiviridae* members revealed by deep sequencing of the *Vanilla* (Orchidaceae) virome. *Archives of Virology*, doi:10.1007/s00705-017-3540-9. * Ho. T., Quito-Avila, D., Keller, K.E., Postman, J.D., Martin, R.R., Tzanetakis, I.E. (2016) Evidence of sympatric speciation of elderberry carlaviruses. Virus Res 215:72-75. * Igori, D., Lim, S., Zhao, F. *et al.* (2016) The complete sequence and genome organization of ligustrum virus A, a novel carlavirus Arch Virol 161: 3593. * James D., Phelan J. (2016) Complete genome sequence of a strain of *Actinidia virus X* detected in *Ribes nigrum* cv. Baldwin showing unusual symptoms. Arch Virol 161:507-511 * Jones, A.T. (1970) Virus from elder. Rep Scott Hort Res Inst 1969:58 * Jones, A.T. (1972) Purification and properties of elderberry latent virus, one of four sap-transmissible viruses obtained from American elder (*Sambucus canadensis* L.). Ann Appl Biol 70:49-58. * Kalinowska, E., Paduch-Cichal, E., Chodorska, M. (2013) First report of Blueberry scorch virus in elderberry in Poland. Plant Dis 97:1515. * Keller, K.E., Mosier, N.J., Thomas, A.L., Quito-Avila, D.F., Martin, R.R. (2015) Identification of two new carlaviruses in elderberry. Acta Hortic 1061:161-164. * Li, Y-S. (2010) Characterization, infectious clone construction and antiserum preparation of *Pitaya virus X*. M.S. thesis. National Taiwan Univ. (In Chinese). * Marais A, Faure C, Candresse T (2016) New insights into Asian prunus viruses in the light of NGS-based full genome sequencing. PLoS ONE 11:e0146420. * Mao, C.H. (2008) Molecular characterization and detection of new *Zygocactus virus X* and *Pitaya virus X* from pitaya. M.S. thesis. National Taiwan Univ. (In Chinese). * Miličić, D., Plavsić, B., Grbelja, J., Erić, Z. (1987) Cherry leafroll virus and Elderberry carlavirus on *Sambucus nigra* L. in South-east Europe. Acta Bot Croat 46:1-8. * Nemchinov, L.G., Grinstead, S.C., & Mollov, D.S. (2017) *Alfalfa virus S*: a new species in the family *Alphaflexiviridae*. PLOS One 12:e0178222. * Oliveira, L.M., Orilio, A.F., Inoue-Nagata, A.K., Nagata, T. and Blawid, R. (2017) A novel vitivirus-like sequence found in *Arracacia xanthorrhiza* plants by high throughput sequencing. Arch Virol 162:2141-2144. * Sabanadzovic, S., Abou Ghanem-Sabanadzovic, N., & Tzanetakis, I. (2011) *Blackberry virus E*: an unusual flexivirus. Arch Virol 156:1665-1669. * Van Lent, J.W.M., Wit, A.J., Dijkstra, J. (1980) Characterization of a carlavirus in elderberry (*Sambucus* spp.). Netherl J Plant Pathol 86:117-134. * Zhao F., Igori D., Lim S., Yoo R.H., Lee S.H., Moon J.S. (2015) Nucleotide sequence and genome organization of *Atractylodes mottle virus*, a new member of the genus *Carlavirus*. Arch. Virol. 160(11):2895-2898 |

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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 three new species in the genus *Potexvirus*, family *Alphaflexiviridae* (Figs. 1 and 2)**

Species demarcation criteria in the family *Alphaflexiviridae* (as defined in the Ninth Report): isolates of distinct species are less than about 72% nt identity (or 80% aa identity) between their respective CP or polymerase (understood in the context of flexiviruses to correspond to the entire ORF1 or replicase) genes.

**Actinidia virus X (AVX) (James & Phelan 2016; KR872420)**

James and Phelan (2016) report the isolation of a potexvirus (RV3124) from black currant plants (Ribes nigrum cv. Baldwin, accession 3124-03D1) showing symptoms of leaf chlorosis and deformity by mechanical transmission into *N. benthamiana*. The complete sequence was determined (GenBank accession number KR872420) from isolated dsRNA and showed 79% nt similarity (and additionally two indels of 72 and 33nt in the replicase gene) to an unpublished near complete virus sequence deposited in GenBank as Actinidia virus X (AVX, accession no. KC568202) determined from *Actinidia chinensis* (Chinese gooseberry; Kiwi fruit), but less than 63% nt similarity to other recognized potexviruses. The genome of RV3124 consists of 6,888 nucleotides (nt) excluding the poly(A) tail. The two available sequences (RV3124 and AVX) share 78-79 % identity at the nt level (89-90 % identity at the aa level) and 83 % nt identity (97 % aa identity) for the polymerase and CP, respectively and therefore should be considered as isolates of the same species, different from recognized species in the genus, which show less than 63% identity. Phylogenetic analysis and evidence of the same recombination event detected upstream of the 3’ terminus of the replicase gene of both virus isolates, providing further evidence of a common origin of the two isolates. The name *Actidinia virus X* is proposed for this new species typified by AVX-RV3124.

**Pitaya virus X (PiVX) (Mao 2008; JF930327)**

A pure virus isolate was obtained from a pitaya plant, confirmed by multiplex RT-PCR, and named as PiVX-P37 (Mao 2008, Li, 2010). This virus isolate produced chlorotic lesions with yellow halo which turned into necrotic lesions on the inoculated leaves of *Chenopodium amaranticolor* at 5-7 days post inoculation. In host range test, PiVX-P37 could locally infect *C. amaranticolor*, *Tetragonia expansa* and *Celosia argentea*, and systemically infected *C. quinoa* and *Hylocereus undatus*. The virions of PiVX are of filamentous shape with 450-500 x 12-13 nm in size as ascertained by transmission electron microscopy, and its capsid protein is about 24-kDa by SDS-polyacrylamide gel electrophoresis analysis. To further study the properties of PiVX-P37, the full-length cDNA clone with a 35S promoter was constructed and its biological activity was tested by inoculating plasmid DNA to *C. amaranticolor* and *C. quinoa*. Five out of seven full-length cDNA clones were infectious on both tested plants, but their levels of infectivity were different. Three of them were completely sequenced, and the sequence identities of these clones are over 99% compared to PiVX-P37. The full-length genome of PiVX-P37 contains 6677 nucleotides without poly(A) tail and five open reading frames. The genome organization of PiVX-P37 is similar to those of other potexviruses. According to sequence comparison of potexviruses, PiVX-P37 is most homologous to Schlumbergera virus X (SVX). The nucleotide sequence identities of RdRp and CP gene between PiVX-P37 and SVX are 70% and 69%, and the amino acid sequence identities are 77% and 81%, respectively; meeting the above-mentioned species demarcating criteria

**Vanilla virus X (VaVX) (Grisoni et al., 2017; MF150240)**

Grisoni et al. (2017) report the identification and whole genome sequencing of a potexvirus from vanilla plants (*V.×tahitensis* accession CRV2148), denominated vanilla virus X (VaVX). The complete sequence was determined by next generation sequencing (NGS) (GenBank accession number MF150240) from isolated dsRNA and from virion-associated nucleic acids obtained from semi-purified particles. The genome shows the typical 5 ORFs potexvirus organization and all ORFs contain the characteristic conserved motifs. The closest relative is *Yam virus X,* and isolates of this species had about 65% overall genome nt similarity with VaVX. The percentage of sequence identity with isolates of known potexvirus species were well below the species demarcation criteria for both the polymerase and coat protein coding regions or encoded proteins. Experimental transmission of the virus to vanilla following mechanical inoculation was obtained. The name *Vanilla virus X* is proposed for this new species.

**Proposal to reassign one existing species (*Blackberry virus E*, currently unassigned in the family *Alphaflexiviridae*) to the genus *Allexivirus*, family *Alphaflexiviridae* and to create three new species in this genus (Figs. 1 and 2)**

Blackberry virus E (BVE, Genbank JN053266) was described in 2011 (Sabanadzovic et al., 2011) as a virus closely related to allexiviruses but lacks the 3’ terminal ORF (ORF7, ca. 15K) and was recently recognized as a member of as a new unassigned species in the family *Alphaflexiviridae*.

Arachis pintoi virus (ApV) was recently described in Colombia (Gutierrez Sanchez et al., 2016 ; Genbank KX058345). Its closest relative is BVE and they have the same genome organization.

Allexiviruses, BVE and ApV lack an AUG translation initiation codon for an ORF4 encoding a TGB3 protein. The potential encoded aa sequence for ORF4 of these two viruses shows however homologies (typical motif [pfam02495](https://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?ascbin=8&maxaln=10&seltype=2&uid=pfam02495) of TGB3 proteins), so that initiation of translation on a non-AUG codons has been hypothesized and may also be the case for ApV.

Alfalfa virus S (AVS) was recently described in alfalfa in Sudan (Nemchinov et al., PLOS One, in press, GenBank KY696659). Its closest relatives are BVE and ApV. It has the same genome organization as these viruses, lacking an ORF7. Its ORF4 contains an AUG start codon and encodes a typical TGB3 protein.

Vanilla latent virus (VLV) is a virus that was recently characterized in vanilla in the Réunion Island by a collaboration of several French teams (Grisoni et al., 2017). Its closest relatives are ApV and AVS. It has the same genomic organization as BVE, ApV and AVS, lacking an ORF7. Similar to AVS, its ORF4 has an AUG initiation codon.

Overall BVE, ApV, AVS and VLV show the same genomic organization, have clear phylogenetic affinities and form a logical ensemble. Their RdRps are 65-72% identical and their CPs are 45-52% identical, suggesting that each represents distinct species within a genus. *[all values given here are amino acid identity].*

**Rationale to include these four viruses in the genus *Allexivirus***

Current members of genus *Allexivirus* infect plant species in the familiy *Liliaceae*, and are transmitted by mites whereas BVE, ApV,VLV and AVS infect plants outside of this family and no vector are currently identified for any of them. Also, BVE, ApV, VLV and AVS do not have an ORF7, while the currently accepted allexiviruses have one. Nevertheless, phylogenetic trees for both CP and RdRp show these four viruses to be evolutionarily closely related to members of the genus *Allexivirus* (see figures 1 & 2). In each case, the basal branch (highlighted in red) has statistically significant bootstrap support.

The identity with allexiviruses is **57.6%-67.7%** for the RdRp and **36.3%-45.6%** for the CP. Only the lower end of the CP comparisons falls below the 42% cut-off for genus discrimination. Nevertheless, these values are significantly higher than the values existing between accepted potexviruses (RdRp : **41.4%-62.3%**; CP: **27.7%-64.8%**). The phylogenetic trees similarly make clear that the accepted diversity within the genome of potexviruses is larger than the diversity that would exist in the genome of allexiviruses if we were to include BVE, ApV, VLV and AVS in this genus.

This is also substantiated by calculating intra-genus average divergence. For the RdRp a value of **65.6% +/- 0.8%** is obtained for allexiviruses if integrating BVE, ApV, AVS and VLV and 46.9% +/- 0.8 for potexviruses. For the CP the corresponding values are **51.5% +/- 1.9%** for the “extended” allexiviruses and 36.5% +/- 1.7% for potexviruses.

Concerning genome organization, the three viruses and the allexiviruses have the same genome organization, particularly with the presence of the additional ORF5 (P40) typical of allexiviruses. The only notable differences are: i) the absence of ORF7 in BVE, ApV, VLV and AVS, 2) the presence of an initiation codon for ORF4 (TGB3) in VLV and AVS. However, the coding potential and conserved motif ([pfam02495](https://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi?ascbin=8&maxaln=10&seltype=2&uid=pfam02495)) typical of TGB3 proteins can be observed in all other viruses (BVE, ApV and allexiviruses) and, assuming an initiation on a non-AUG codon, all viruses potentially encode a small typical TGB3 protein.

Given the similar genome organizations, the sharing of the typical allexivirus ORF5 (P40), phylogenetic affinities and the fact that the diversity within the genus *Allexivirus*, extended to include these four novel viruses, would still be lower than that seen in the genus *Potexvirus*, justify an assignment of BVE, ApV, VLV and AVS in the genus *Allexivirus*.

Regarding the absence of ORF7 in the four viruses proposed to typify novel species, several precedents exist for which there is variability in number of ORFs (as well as in transmission properties) between members of a genus within the order *Tymovirales*, e.g. the genus *Trichovirus* in the sister *Betaflexiviridae* family, with most members lacking the terminal ORF and having no known vector and isolates of the species *Peach mosaic virus* and *Cherry mottle leaf virus* having a terminal ORF and mite vectors.

***Blackberry virus E* (BVE) (Sabanadzovic et al., 2011; JN053266)**

*Blackberry virus E* is currently an unassigned species in the family *Alphaflexiviridae* and we propose to assign it to the genus *Allexivirus* .

**Arachis pintoi virus (ApV) (Gutierrez Sanchez et al., 2016; KX058345)**

In a NGS approach of total RNA isolated from a pool of symptomatic *Arachis pintoi* plants, Gutierrez Sanchez et al. (2016) obtained two distinct contigs that share 85% nucleotide sequence identity. Both sequences show the highest overall nt sequence identity (both 63%) to an isolate of the species *Blackberry virus E* (currently unassigned in the family *Alphaflexiviridae*), followed by less than 60% identity to isolates of *Garlic virus A* (GarVA, genus *Allexivirus*). The deduced genome organization (identical for both contig variants) resembles that of allexiviruses with the difference that it lacks the 3’end-proximal ORF (NABP), which is comparable to the genome organization of BVE. Similar to BVE and allexiviruses, it lack an ATG codon for ORF4, although the ORF coding capacity and sequence is conserved. For the ORF1 encoded replicase typical Mtr, Hel and RdRp motifs can be predicted. Both variants show the highest aa sequence identity to BVE (72% in the ORF1 protein), followed by 68% to two isolates of GarVA (the two isolates share 94% aa identity). For the ORF5 encoded putative coat protein, the most closely related virus is again BVE, (50 to 52% aa sequence identity with the Arachis pintoi virus variants) followed by 39 to 42% identity with the CPs of allexiviruses. Besides the lack of ORF6 and the sequence identity values, the relationship to BVE is also supported by the phylogenetic trees provided by Guiterrez Sanchez et al. (2016). However, both replicase and CP sequence identity values are below the species demarcation threshold of 80% aa sequence identity. Other information, e.g. regarding ApV transmission is not available. The symptoms observed in the original Arachis samples cannot be linked to ApV due to the identification of additional poty- and luteoviruses. Therefore we propose to create a new species in the genus *Allexivirus* of the family *Alphaflexiviridae*, with the proposed name of *Arachis pintoi virus*.

**Alfalfa virus S (AVS) (Nemchinov et al., 2017; KY696659)**

In a NGS approach of total RNA isolated from alfalfa plants from Sudan, Nemchinov et al., (2017) assembled and then completed the genome sequence of a virus with highest overall nt sequence identity (54-55%) to the isolate of Arachis pintoi virus variants A and B (see above), an isolate of *Blackberry virus E* (unassigned in the family *Alphaflexiviridae*) (51%), followed by 50% identity to the exemplar isolate of the type member of the genus *Allexivirus*, *Shallot virus X*. The deduced genome organization resembles that of allexiviruses, with the difference that it lacks the 3’end-proximal ORF (NABP), a situation comparable to that observed in BVE and ApV. Contrary to BVE, ApV and allexiviruses, a potential AUG codon exists for ORF4, which encodes a typical TGB3 protein. For the ORF1-encoded replicase, typical Mtr, Hel and RdRp motifs can be predicted. The RdRp of AVS show the highest aa sequence identity with ApV (69%), followed by BVE (63%) and by GarVA (61% aa identity). For the ORF5 encoded putative coat protein, the most closely related virus is ApV sharing 46% aa CP sequence identity, followed by BVE (45%) and allexiviruses (36-40%). Besides the lack of ORF7 and the sequence identity values, the relationship to BVE and ApV is also supported by the phylogenetic trees provided by Nemchinov et al. (2017). However, both replicase and CP sequence identity values are below the species demarcation threshold of 80%. Transmission electron microscopic observations of the infected tissues showed the presence of filamentous particles similar to allexiviruses in their length and appearance. Other information, e.g. regarding AVS transmission is not available. We propose the creation of a new species named *Alfalfa virus S* in the genus *Allexivirus* of the family *Alphaflexiviridae*.

**Vanilla latent virus (VLV) (Grisoni et al., 2017; MF150239)**

In a NGS approach of double stranded RNAs from vanilla plants from the Réunion Island, Grisoni et al. (2017) assembled and then completed the genome sequence of a virus with highest overall nt sequence identity to BVE, ApV and AVS followed by members of the genus *Allexivirus*. The deduced genome structure resembles that of allexiviruses, with the difference that it lacks the 3’end-proximal ORF (NABP), organization comparable to that seen in BVE, ApV and AVS. Similar to AVS, but contrary to BVE, ApV and allexiviruses, a potential AUG codon exists for ORF4, which encodes a typical TGB3 protein. For the ORF1 encoded replicase, typical Mtr, Hel and RdRp motifs can be predicted. The RdRp of VLV show the highest aa sequence identity with ApV (69%), followed by BVE (65%), GVA (64%) and AVS (64%). For the ORF5-encoded putative coat protein, the most closely related virus is again BVE (49% aa sequence identity) followed by ApV (47%), AVS (45%) and allexiviruses (37-39%). Besides the lack of ORF7 and the sequence identity values, the relationship to BVE is also supported by the phylogenetic trees provided by Grisoni et al. (2017). However, both replicase and CP sequence identity values are below the species demarcation threshold of 80%. Other information, e.g. regarding its transmission is not available, but VLV has been shown to be experimentally mechanically transmissible between vanilla plants. Allexiviruses are transmitted by mites and the vector of BVE is unknown. The symptoms observed in the original vanilla samples cannot be linked to VLV due to the identification of additional viruses. We propose the creation of a new species named *Vanilla latent virus* in the genus *Allexivirus* of the family *Alphaflexiviridae*.

**Proposal to create six new species in the genus *Carlavirus*, family *Betaflexiviridae*, subfamily *Quinvirinae* (Fig. 3)**

Species demarcation criteria in the family *Betaflexiviridae* are identical to those described above for the family *Alphaflexiviridae*

**Yam latent virus (YLV) (Zou et al., 2011; KJ789130)**

In 2011, Zou et al. identified a putative new carlavirus in the host plant belonging to *Dioscorea opposita* grown in Shandong province in China. The only information that is publicly available is a GenBank entry under acc. no. KJ789130. The isolate has a typical carlavirus genome organization as well as ORF size. The ORF1 encoded replicase shows the typical Mtr, P-Pro, Hel and RdRp motifs but lacks the AlkB domain. Regarding species demarcation, the yam latent virus (YLV) encoded replicase shows the highest aa sequence identity (62%) to an isolate of *Hop mosaic virus*. The ORF2-4 encoded TGB1-3 proteins. The coat protein shows the highest aa sequence identity (68%) to an isolate of *Aconitum latent virus*. Both replicase and CP sequence identity values are below the species demarcation threshold of 80%. As known for other carlaviruses, the ORF6 encoded NABP includes an N-terminal signal peptide and a “zinc finger” motif. Information regarding other criteria relevant for species demarcation (host range, serological reactions etc.) are not available.

There is some information available about a carlavirus named “Chinese yam necrotic mosaic virus” (VIDE Database) occurring in yam. This virus was first reported from Japan by Fukumoto & Tochihara in 1978 to occur in “*Dioscorea batatas”*, which is an old synonym of *Dioscorea opposita*, hence the same species. Due to missing reference material/sequence information it cannot be checked whether the proposed “yam latent virus” is related to it or not.

**Sambucus virus C-E (SVC-E) (Ho et al., 2016; KJ572560- KJ572564)**

In 2016, Ho et al. published the full genome sequences of five carlaviruses infecting elderberry (*Sambucus* spp.) in a germplasm collection in the United States, although two sources were originally obtained from Japan or Uzbekistan. The five carlaviruses were found in various combinations, with only one of the original source plants having a single infection of a carlavirus; four samples were additionally infected with either one or two viruses belonging to other genera. The five viruses were found to separate into two separate clades in phylogenetic trees based on either the replication protein or coat protein amino acid sequences, with each clade having high bootstrap support; these clades were further distributed between two major branches within the genus *Carlavirus*. The five viruses were provisionally named elderberry virus A through elderberry virus E (ElVA – ElVE), and the sequences are available under GenBank accession numbers KJ572560 – KJ572564, and NC\_029085 – NC\_029089 respectively.

All five viruses have genomes with an organization typical of other carlaviruses – a large replication protein, followed by a triple gene block, coat protein, and nucleic acid binding protein, and an overall genome length of 8,540 – 8,628 nt excluding the poly(A) tail. The clade of three viruses (ElVA, ElVB, and ElVD) has a replicase including methyl transferase; 2-oxyglutarate and Fe(II)-dependent oxygenase; carlavirus endopeptidase; helicase; and RdRp domains; the clade of two viruses (ElVC, ElVE) has all these domains, but also includes an ovarian tumour-like cysteine protease domain (c.95 residues) upstream of the carlavirus endopeptidase domain.

The genomes of each of the five viruses share 42-66% nt identity to each other, with 40-58% nt identity to other reference carlavirus genomes; the replicase proteins share 40-71% aa identity to each other, and 36-58% to accepted members of the genus *Carlavirus*. These values are clearly below the accepted species discrimination level of 80% aa identity for both REP and CP; **however the CPs of ElVA and ElVB share 89% aa identity**, compared to 34-79% aa identity between other elderberry carlaviruses, and 25-63% to any other carlaviruses (see Supplementary materials to Ho et al., 2016). **Thus the CPs of ElVA and ElVB are not clearly discriminated** although the replicase protein is clearly discriminated by the 80% criteria, and all other ORFs have ≤68% aa identity, and ≤57% aa identity to the equivalent ORFs of any other carlavirus. The genome ‘backbones’ of ElVA and ElVB are thus clearly distinguished from each other, with only 66% nt identity (Ho et al., 2016), although one of the two may have gained the CP from the other by recombination; currently available information does not permit identification of ElVA or ElVB as either the ‘parental’ isolate or as a recombinant. Due to the divergence of the majority of the genome, it is not appropriate to consider ElVA and ElVB as members of the same species, regardless of the similarity of their CP genes. However, throughout the family *Betaflexiviridae*, the current sequence-based species demarcation criteria are less than about 72% nucleotide identity (or 80% amino acid identity of encoded proteins) in the CP **or** polymerase genes. With the established ‘**CP or polymerase**’ distinction, ElVA and ElVB can be accepted as representatives of distinct carlavirus species without requiring any change to the species demarcation criteria for either the genus *Carlavirus* or family *Betaflexiviridae*. Nevertheless, the flexiviridae study group majority preferred to wait with classifying these two viruses until further information became available or a consensus could be reached on how to deal with such cases of apparent recombination.

There are multiple prior reports of carlaviruses from elderberry, including a previously recognized species, now abolished, name elderberry symptomless virus (synonym elderberry carlavirus). There is no molecular data for elderberry symptomless virus, and as pointed out by Ho et al. (2016), it may have been a mixed infection; they also note that another recognized carlavirus, blueberry scorch virus, is also known to infect elderberry, and has been reported to be symptomless in Poland (Kalinowska et al., 2013). Other reports of elderberry carlaviruses include ‘elderberry carlavirus-North American isolate’ (Ellis et al., 1992) and ‘elderberry carlavirus-European isolate’; antibodies against ‘elderberry carlavirus-North American isolate’ do not react with the new viruses elderberry virus C or elderberry virus D, which fall into different clades (Keller et al., 2015; Ho et al., 2016). The ‘elderberry carlavirus-European isolate’ was described from *Sambucus racemosa* and *S. nigra* in the Netherlands (Van Lent et al., 1980) and found to be serologically related to the elderberry virus A of Jones (1970, 1972). An elderberry carlavirus has also been reported from *S. nigra* in South-east Europe (Miličić et al., 1987).

In view of these prior reports of carlaviruses for which no molecular data are available, and apparently no cultures extant, as well as the prior use of the name ‘elderberry virus A’, we feel that it is appropriate to fully disambiguate the five new, fully molecularly described carlaviruses described by Ho et al. (2016). We therefore propose that the viruses described as elderberry virus C to elderberry virus E by Ho et al. (2016) should be recognized as isolates of three new carlavirus species under the names *Sambucus virus C* to *Sambucus virus E*, respectively, represented by the following GenBank accession numbers:

**Sambucus virus C** (elderberry virus C of Ho et al., 2016) – KJ572562, NC\_029087

**Sambucus virus D** (elderberry virus D of Ho et al., 2016) – KJ572563, NC\_029088

**Sambucus virus E** (elderberry virus E of Ho et al., 2016) – KJ562564, NC\_029089

We recommend waiting with classifying elderberry virus A and B until further information is available about the frequency and extent of CP recombination events in this group of viruses or a general consensus can be reached on how to deal with classification of such recombinant events.

**Atractylodes mottle virus (AtrMV) (Zhao et al., 2015; KR349343)**

In 2015 Zhao et al. published an annotated sequence record in which a new carlavirus virus, Atractylodes mottle virus (AtrMV) is described. The source is *Atractylodes macrocephala*, an East Asia grown medicinal herb (Compositae) showing mottling symptoms. The new virus was originally discovered by analysis of RNASeq data, in which, among virus-like contigs obtained, three contigs had partial homology with the carlavirus Chrysanthemum virus B. The complete genome of the new virus was neatly obtained (KR349343) through RT-PCR performed on total RNA extracts from *A. macrocephala* plants. The full sequence (8881nt in the NCBI record) showed a typical carlavirus genome organization (5’UTR, 6 ORFs and 3’UTR) and deduced ORF products show similarity to carlavirus viral proteins; the polymerase and coat protein show the highest aa sequence identity (58 and 65%, respectively) to isolates of *Chrysanthemum virus B* (recognized carlavirus). Both sequence identity values are well below the species demarcation threshold of 80% and we propose the creation of a new species termed *Atractylodes mottle virus* in the genus *Carlavirus*.

**Ligustrum virus A (LiVA) (Igori et al., 2016; KX000914)**

The complete genome sequence of Ligustrum virus A (LiVA) from a *Ligustrum obtusifolium* Sieb. & Zucc. plant was determined by Igori et al. (2016). The genomic RNA has 8,525 nucleotides, excluding the poly(A) tail, and consists of six open reading frames typical of members of the genus *Carlavirus*, family *Betaflexiviridae*. Phylogenetic analysis of the viral replicase and coat protein (CP) indicated that LiVA is closely related to daphne virus S and Helenium virus S. The replicase and CP of LiVA shared 44.73–52.35 % and 25.39–62.46 % amino acid identity, respectively, with those of other carlaviruses. Based on these results we propose the creation of a new species termed *Ligustrum virus A* in the genus *Carlavirus*.

**Proposal to create one new species in the genus *Foveavirus*, family *Betaflexiviridae,* subfamily *Quinvirinae* (Fig. 3)**

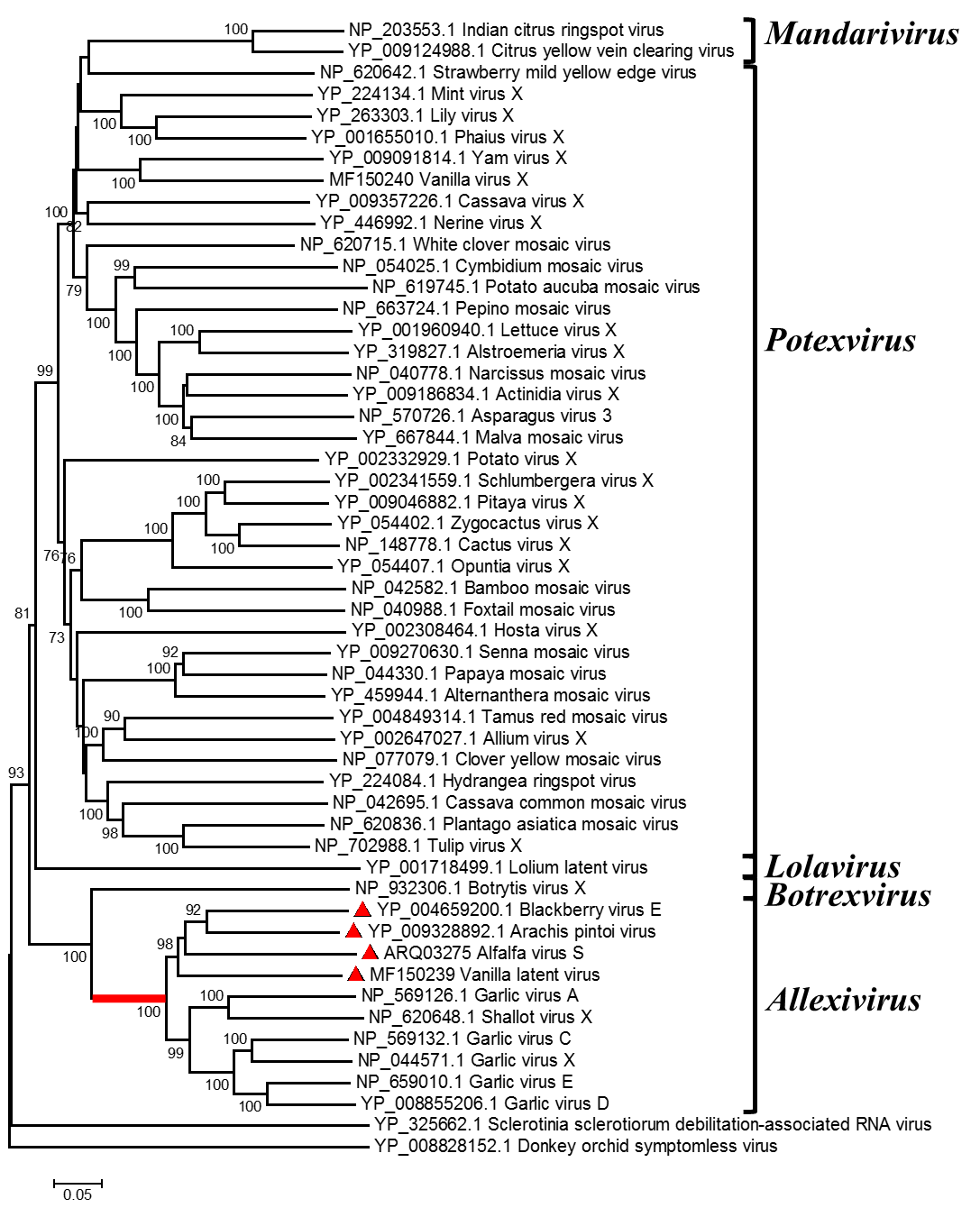
**Asian prunus virus 2 (APV2) (Marais et al., 2016; KT893294)**

Completion of the genomic sequences and analysis of the phylogenetic relationships between Asian prunus virus 1 (APV1) and Asian prunus virus 2 (APV2) isolates has recently been published (Marais et al., 2016). This allows a clarification the taxonomic status of APV2 and to propose it as a representative of a new species in the genus *Foveavirus*. Throughout the family *Betaflexiviridae*, sequence-based species demarcation criteria are less than about 72% nucleotide identity (or 80% amino acid identity of encoded proteins) in the CP or polymerase genes. Viruses from different genera usually have less than about 45% nucleotide identity in these genes (Adams et al., 2012). Genome structure and phylogenetic analyses show that the closest characterized virus to APV2 is APV1, a member of the genus *Foveavirus*. In particular, the genomes of the two viruses are essentially colinear and show the same genetic organization, typical of foveaviruses (Figure 1 and Table 1 in Marais et al., 2016). The nucleotide sequence of the polymerase genes of APV1 and APV2 are 70.2-71.3% identical while the encoded proteins are 79.1-80.1% identical. The corresponding values for their CP genes are of 62.5-64.5% nucleotide sequence and 63.2-65% amino acid sequence identity, respectively (Table 2 in Marais et al., 2016). Although these values fall close to (but largely below) the species demarcation criteria for the polymerase gene, they clearly fall outside the species demarcation criteria when it comes to the CP gene. So far both viruses have only been detected infecting *Prunus* sp. species (peach, *P. mume*) in Asia (Japan, China, Korea), or found elsewhere (USA) in *Prunus* material of Asian origin. In conclusion, APV2 has a genomic organization similar to membersof the genus *Foveavirus*, but its sequence divergence values for CP and polymerase genes fall below the species demarcation criteria, suggesting the creation of a new species named *Asian prunus virus 2*.

**Proposal to create one new species in the genus *Vitivirus*, family *Betaflexiviridae,* subfamily *Trivirinae* (Fig. 4)**

**Arracacha virus V (Oliveira et al., 2017; KY392781)**

The complete genome of a new vitivirus-like sequence was identified by high throughput sequencing of RNA isolated from a preparation enriched for virus like particle from arracacha (*Arracacia xanthorrhiza*) plants from Brazil and was validated by Sanger sequencing (KY392781; NC\_034264, Oliveira et al., 2017). The genomic organization of the new putative vitivirus resembles that of grapevine virus B (GVB) and grapevine virus D (GVD). The putative coat protein showed 41 to 49% identity with similar proteins of known vitiviruses, while the RNA-dependent RNA polymerase shared 52 to 55% aa identity with those encoded by grapevine vitiviruses. Independently de Souza et al. (submitted) identified another isolate of the same virus (KY\_451036) in Peruvian arracacha germplasm and which showed 82% nt and 92% aa identity to the sequence of NC\_034264 from Brazil. In both isolates the ORF encoding a 20kDa protein initiates with a GUG codon. Souza et al. also found the *Solanum phureja* (native diploid yellow potatoes) plants from Colombia also infected by this virus. Based on the demarcation criteria for the genus *Vitivirus*, the virus described in these works, represent an isolate of a novel species within the genus *Vitivirus*, for which the name *Arracacha virus V* is proposed.



**Figure 1.** Phylogenetic tree of aligned replicase amino acid sequences of viruses in the family *Alphaflexiviridae*. Viruses proposed here as new members of the genus *Allexivirus* are marked by a red triangle, whereas suggested new potexvirus species are marked with a blue triangle. The branch to members of the genus *Allexivirus* is shown in red.

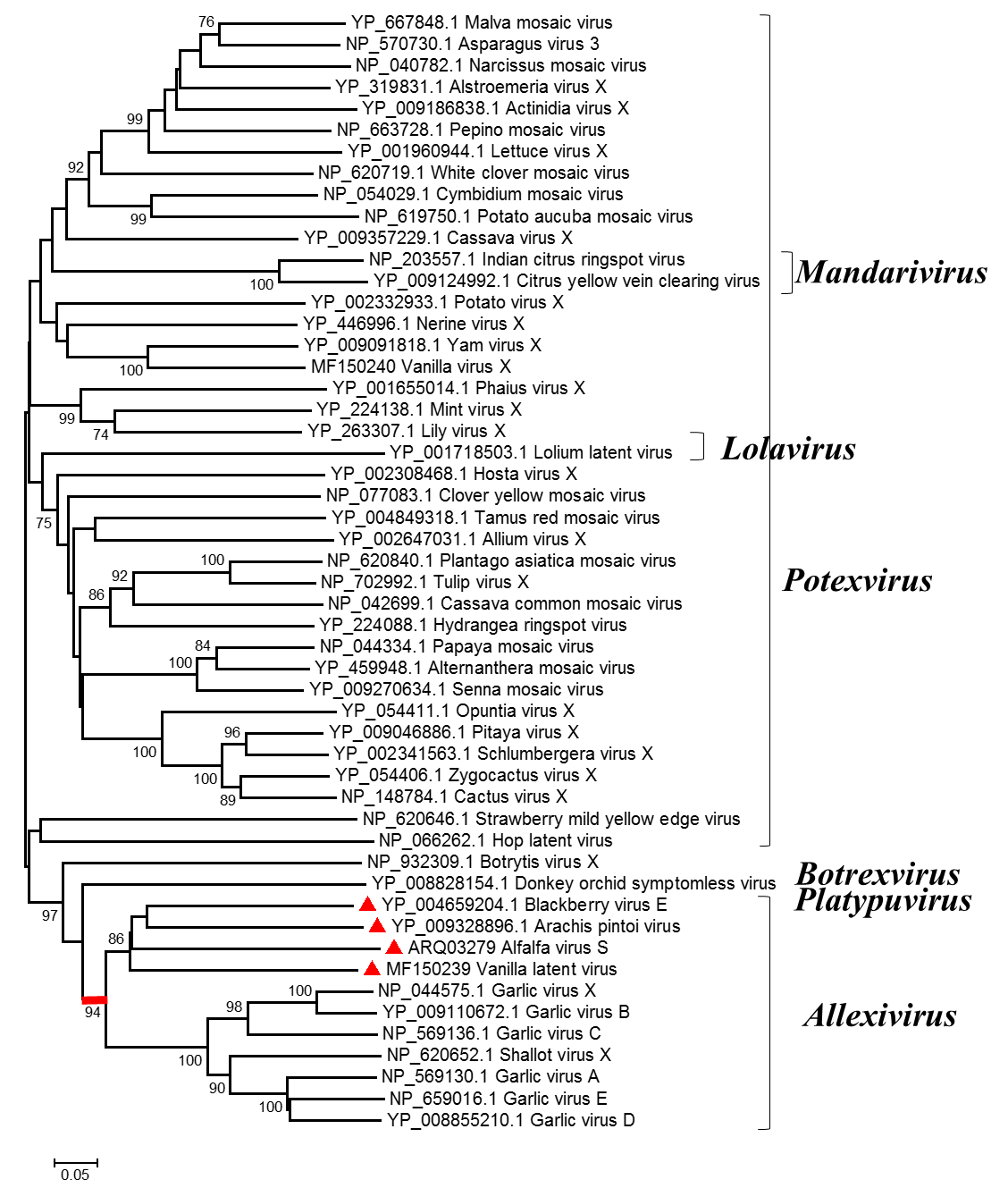


Figure 2. Unrooted phylogenetic tree of aligned coat protein amino acid sequences of viruses in the family *Alphaflexiviridae*. Viruses proposed here as new members of the genus *Allexivirus* are marked by a red triangle whereas a newly proposed potexvirus species are marked in blue. The branch to members of the genus *Allexivirus* is shown in red.

*Foveavirus*

unassigned

Unassi

*Carlavirus*

unassigned

**Figure 3.** Phylogenetic Neighbour Joining tree of aligned replicase amino acid sequences of viruses in the sub-family *Quinvirinae* within the family *Betaflexiviridae*. Viruses proposed here as a member of new species are shown in red in the genera *Carlavirus* and *Foveavirus*, and the two viruses that are recombinant for their CP gene and have not yet been classified are indicated in orange. The branch to members of the genus *Robigovirus* has been collapsed for clarity. Hardenbergia virus A was used as an outgroup.

Narcissus common latent virus AM158439

Hop mosaic virus EU527979

**Yam latent virus KJ789130**

Hop latent virus AB032469

Aconitum latent virus AB051848

Potato virus M D14449

Hippeastrum latent virus DQ098905

Cowpea mild mottle virus KC774019

Daphne virus S AJ620300

**Ligustrum virus A KX000914**

**Atractylodes mosaic virus** **KR349343**

Chrysanthemum virus B AB245142

Phlox virus B EU162589

Gaillardia latent virusV KJ415259

Phlox virus S EF492068

Potato latent virus EU433397

Potato virus H HM584819

**Sambucus virus C KJ572562**

**Sambucus virus E KJ572564**

Potato rough dwarf virus JQ245696

Potato virus S AJ863510

Potato virus P EU020009

Blueberry scorch virus AY941198

Hydrangea chlorotic mottle virus EU754720

Ligustrum necrotic ringspot virus EU074853

Lilly symptomless virus AJ516059

Kalanchoe latent virus FJ531634

Mirabilis jalapa mottle virus JN039374

Red clover vein mosaic virus FJ685618

Garlic latent virus AJ292226

Butterbur mosaic virus AB517596

Coleus vein necrosis virus EF527260

Garlic common latent virus JF320810

Helleborus net necrosis virus AB623047

Poplar mosaic virus AY505475

**Sambucus virus D KJ572563**

**Elderberry virus A KJ572560**

**Elderberry virus B KJ572561**

NeLV AM182569

MYaV LC224308

Sweet potato chlorotic fleck virus AY461421

Sweet potato C6 virus JX212747

Asian prunus virus 1 FJ824737

**Asian prunus virus 2 KT893294**

Rubus canadensis virus 1 JX277553

Grapevine rupestris stem pitting associated virus AF026278

Peach chlorotic mottle virus EF693898

Apple stem pitting virus D21829

Apricot latent virus HQ339956

Sugarcane striate mosaic associated virusAF315308

*Robigovirus*

Banana mild mosaic virus AF314662

Hardenbergia virus A HQ241409

100

100

100

100

100

100

100

100

100

86

99

99

100

100

100

71

99

98

69

97

100

98

100

100

71

88

100

100

100

97

96

72

98

100

99

87

90

79

72

100

0.2

Grapevine virus F JX105428

Grapevine virus A X75433

Actinidia virus B JN427015

Grapevine virus B X75448

**Arracacha virus V KY392781**

Grapevine virus E AB432910

Agave tequilana leaf virus KY190215

Vitivirus

Tepovirus

Citrivirus

Chordovirus

Prunevirus

Trichovirus

Divavirus

BanMMV AF314662

100

100

100

100

100

85

100

65

100

100

98

99

62

44

87

61

39

0.2

**Figure 4.** Phylogenetic Neighbour Joining tree of aligned replicase amino acid sequences of viruses in the sub-family *Trivirinae* withing the family *Betaflexiviridae*. Arracacha virus V proposed here as a member of new species is shown in red. The branch to members of other genera in the sub-family have been collapsed for clarity.