This Word module should be used for all taxonomic proposals.

Please complete **Part 1** and:

either **Part 3** for proposals to create new taxa or change existing taxa

or **Part 2** for proposals of a general nature.

Submit the completed Word module, together with the accompanying Excel module named in Part 3, to the appropriate ICTV Subcommittee Chair.

The Word module explains and justifies your proposal. The Excel module is a critical document that will be used to implement the proposed taxonomic changes once they are approved and ratified. If proposals presented in the Word module are not presented accurately in the Excel module, the taxonomic changes cannot proceed.

For guidance, see the notes written in blue, below, and the Help Notes in file Taxonomic\_Proposals\_Help\_2019.

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

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| **Code assigned:** | ***2019.023P*** | |  | |
| **Short title:** Create seven new species in the family *Endornaviridae* | | | | |
|  | | | | |
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| **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) | | *Endornaviridae* Study Group | | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | | |
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| Date first submitted to ICTV: | | | June 1, 2019 | |
| Date of this revision (if different to above): | | |  | |
| **ICTV-EC comments and response of the proposer:** | | | | |
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**Part 2:** **NON-STANDARD**

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

| **Text of proposal:** |
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**Part 3:** **PROPOSED TAXONOMY**

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| **Name of accompanying Excel module:** 2019.023P.A.v1.Endornaviridae\_7sp | |
| **Proposal to create seven new species in the family *Endornaviridae* (five in the genus *Alphaendornavirus* and two in the genus *Betaendornavirus*)**  Family *Endornaviridae* contains capsidless viruses with ssRNA genomes infecting plants, fungi and oomycetes that are efficiently transmitted vertically and, in most cases, cause no visible effects in the host (Valverde et al., 2019). At present, members of this family have been classified in 24 species belonging to one of the two genera, *Alphaendornavirus* and *Betaendornavirus*, depending upon genome size, presence/absence of certain functional domains in the virus-encoded polyprotein, and evolutionary history of the viral RNA-dependent RNA polymerase (RdRP).  Current criteria for species demarcation in the genera *Alphaendornavirus* and *Betaendornavirus* of the family *Endornaviridae* are:   * Overall nt sequence identity less than 75% * Differences in host   Based upon results of our own studies and an extensive bibliographic review, we propose the creation of seven new species (five in the genus *Alphaendornavirus* and two in the genus *Betaendornavirus*) to classify recently described viruses with properties resembling members of the family *Endornaviridae*.  **Genus *Alphaendornavirus***   1. ***Agaricus bisporus alphaendornavirus 1***   Agaricus bisporus endornavirus 1 (AbEV1), isolate 2990, is a recently characterized endornavirus associated with mushroom virus X disease of the cultivated mushroom *Agaricus bisporus* (Deakin et al., 2017). AbEV1-2990 has a 12,730 nt long genome (GenBank KY357509), of which >95% putatively code for a protein composed of 4,217 residues with an estimated molecular mass of 478.2 kDa containing three identifiable functional domains: viral helicase, glycosyl transferase and RNA-dependent RNA polymerase (Figure 1). Phylogenetically, AbEV1 forms a distinct lineage among alphaendornaviruses (Figure 2). Furthermore, differences in nucleotide sequence content with all officially recognized endornaviruses exceed those recommended for species demarcation.  In summary, AbEV1-2990 is a novel endornavirus that we propose to be classified in a newly established species for which we propose name *Agaricus bisporus alphaendornavirus 1*.   1. ***Cluster bean alphaendornavirus 1***   A large dsRNA, approx. 13-14 kbp in size, was identified in tissue of a symptomless cluster bean (*syn*. guar; Cyamopsis tetragonoloba) PI line 593049 and used as a template for cDNA library preparation and custom Illumina MiSeq sequencing. BLASTx comparisons with available data showed similarity of several contigs obtained by assembling raw sequence data with few recognized endornaviruses. The complete sequence of the putative endornavirus, named cluster bean endornavirus 1 isolate isolate 593049 (CBEV1-593049), consists of 12,895 nt (GenBank MG764084) and contains an open reading frame coding for a polyprotein of 4,207 aa in length (Alcalá-Briseño et al., 2018). Computer-assisted analyses of the putative polyprotein sequences revealed the presence of three conserved functional domains: methyltransferase (MTR), helicase (HEL), and RNA-dependent RNA polymerase (RdRP)(Figure 1). A BLASTx search using the polyprotein sequence showed that Hordeum vulgare endornavirus is the closest CBEV1 relative among currently recognized endornaviruses, sharing 23.5% aa content. A phylogenetic tree constructed using the RdRp domain placed it in a clade with alphaendornaviruses (Figure 2). Low level of identities shared with recognized members of the family *Endornaviridae* strongly suggest that CBEV1 is a distinct and yet not classified endornavirus.  Therefore, based upon the above-reported data, we propose that cluster bean endornavirus 1 (CBEV1) represents a new species in the genus *Alphaendornavirus*, for which we propose the name *Cluster bean alphaendornavirus 1,* with CBEV1-593409 as an exemplar isolate.   1. ***Helianthus annuus alphaendornavirus***   A new endornavirus has recently been discovered in two imported varieties of sunflower (*Helianthus annuus*) during routine check-up for possible quarantine viruses in China applying high throughput sequencing of small RNAs (Liu et al., 2018). Contigs assembled from raw sequence data of two varieties, X3939 and SH1108, showed similarities with endornavirus sequences available in GenBank.  Complete sequencing, as well as phylogenetic analysis of viral RdRP, showed that this putative new endornavirus, named Helianthus annuus alphaendornavirus, isolate BJ (HaEV-BJ), has a monocistronic genome of 14,662 nt (GenBank MF362666) ending with eight cysteine residues (C8). A large ORF encodes a deduced 4,867 aa long polyprotein with three identifiable functional domains: helicase, UDP-glycosyltransferase and RNA-dependent RNA polymerase (Figure 1). As other endornaviruses, HaEV is highly transmissible via seeds.  Based upon its typical endornavirus genome organization, along with limited levels of identities and phylogenetic relationships (Figure 2) with extant alphaendornaviruses, we propose the creation of a new species in the genus *Alphaendornavirus* with the proposed name *Helianthus annuus alphaendornavirus 1,* typified by the isolateHaEV-BJ.   1. ***Phaseolus vulgaris alphaendornavirus 3***   The genome of a third distinct endornavirus from common bean (*Phaseolus vulgaris*), named Phaseolus vulgaris endornavirus 3, was recently characterized from cv. ‘Clouseau’ (PvEV3-Clou) using high-throughput sequencing (Okada et al., 2018). The complete sequence of PvEV3-Clou is 15,205 nt long (GenBank MG242064) and contains a single ORF coding for a polyprotein of 4,932 aa, preceded and followed by untranslated regions of 344 nt and 62 nt, respectively. The putative PvEV3-Clou polyprotein contains identifiable domains of helicase, peptidase C97, glycosyltransferase of the GTB-type, and RNA-dependent RNA polymerase (Figure 1).  The polyprotein shares 31% amino acid identity with the counterpart encoded by its closest relative, Hordeum vulgare endornavirus. Phylogenetic analyses of viral RdRP place PvEV3-Clou in a clade with members of the genus *Alphaendornavirus* (Figure 2). In a RT-PCR-based survey, performed with PvEV3-specific primers, this virus was detected in cultivated and wild *P. vulgaris* genotypes either in single and/or mixed infections with PvEV1 and PvEV2. The natural infection of common bean with three distinct endornaviruses is unprecedented in plant-endornavirus systems.  Based upon the results of this study we propose the creation of a new alphaendornavirus species named *Phaseolus vulgaris alphaendornavirus 3*, with PvEV3-Clou as its exemplar isolate.   1. ***Rhizoctonia solani alphaendornavirus 2***   The genome of a new endornavirus referred to as Rhizoctonia solani endornavirus 2 (RsEV2) was identified from the fungal isolate “Illinois1” in a study aimed to characterize the virome of five major phytopathogenic fungi applying high-throughput sequencing-based metatranscriptomics (Marzano et al., 2016). The genome sequence of RsEV2-Illinois1 is 15,850 nt long (GenBank KT823701) and codes for a single long polyprotein with the following functional domains (from N to C terminus): phospholipase A2-like domain, viral helicase, M phase phosphoprotein 10 related domain, and viral RdRP (Figure 1). Such genome organization is distinct from recognized members of the family *Endornaviridae*. In phylogenetic trees, RsEV RdRP belongs to a sister lineage to that of HaEV1 (Figure 2). Direct sequence comparisons of RsEV2-Illinois1 with 24 recognized endornaviruses showed limited levels of similarity (below 50%).  Based upon these results, we propose the creation of a new species with the proposed name *Rhizoctonia solani alphaendornavirus 2* typified by RsEV2-Illinois1 as an exemplar isolate.  **Genus *Betaendornavirus***   1. ***Rosellinia necatrix betaendornavirus 1***   A novel endornavirus named Rosellinia necatrix endornavirus 1 (RnEV1) was identified in three of 365 *R. necatrix* isolates, collected under greenhouse conditions and tested for the presence of dsRNA viruses (Yaegashi and Kanematsu, 2016). The complete genome of RnEV1 isolate W1141 (RnEV1-W1141) consists of 9,639 nt and contains a single large ORF putatively coding for a 3,148 aa long polyprotein (GenBank LC076696).  BLASTp analyses of the RnEV1-W1141 polyprotein identified three functional domains: methyl transferase (MTR), viral helicase (HEL) and RNA-dependent RNA polymerase (RdRP) (Figure 1). Phylogenetic analyses of the viral RdRP domain showed that RnEV1-W1141 belongs to the genus *Betaendornavirus* (Figure 2). RnEV1-W1141 had no detectable biological impact on the host fungus.  Pairwise comparisons with recognized betaendornaviruses showed that RnEV1-W1141 shares sequence identities below the species demarcation cut-off and therefore belongs to a novel species in the genus *Betaendornavirus*, for which we propose the name *Rosellinia necatrix betaendornavirus.*   1. ***Sclerotinia minor betaendornavirus 1***   Sclerotinia minor endornavirus 1 has been isolated from the hypovirulent *S*. *minor* strain LC22 (SmEV1-LC22; Yang et al., 2018). The genome of SmEV1-LC22 is 12,626 nt long (GenBank MG255170) containing a single, large open reading frame (ORF), and lacks a site-specific nick described in some endornaviruses. The deduced 4,020 aa-long polyprotein contains viral methyltransferase (MTR), cysteine-rich region (CRR), putative DEXDc, viral helicase (HEL), and RNA-dependent RNA polymerase (RdRP) domains (Figure 1). In phylogenetic trees constructed with aa sequences of the viral RdRP, SmEV1-LC22 grouped with extant betaendornaviruses (Figure 2).  SmEV1 was readily transmitted horizontally via hyphal contact to several other isolates of *S. minor* inducing a hypovirulent phenotype. Furthermore, SmEV1-LC22 was also vertically transmitted through sclerotia. Pairwise comparisons of complete SmEV1-LC22 genome sequences with recognized members of the family showed that it shares identity levels below the currently valid species demarcation threshold.  In conclusion, we propose to recognize a new species in the genus *Betaendornavirus* named *Sclerotinia minor betaendornavirus 1* with SmEV1-LC22 being its exemplar isolate. | |

| **References:** |
| --- |
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**Figure 1.** Genome organization of viruses representing new alpha- and betandornavirus species proposed to created. The genome of each virus is monocistronic and contains a single, long open reading frame depicted as a box. Lines show short untranslated regions (UTRs). Functional domains identified in each virus encoded protein are reported as dark grey boxes.



**Figure 2.** Phylogenetic tree based on amino acid sequences of viral RNA-dependent RNA polymerases. The tree was inferred using the maximum likelihood (ML) method based on the best-fit amino acid substitution model. Endornavirus lineages belonging to the genus *Alphaendornavirus* are shaded green, while those of the genus *Betaendornavirus* are shaded yellow. Percentages of bootstrap support out of 1000 iteration are reported on main branching points. The analysis was implemented in MEGA7 (Kumar et al., 2016). Names, acronyms and GenBank accession numbers of genome sequences for viruses used to construct trees are: Agaricus bisporus endornavirus 1 (AbEV, KY357509), Alternaria brassicicola endornavirus 1 (AbEV1, KP239989), Basella alba endornavirus 1 (BaEV1, AB844264), Botrytis cinerea endornavirus 1 (BcEV1, KU923747), bell pepper endornavirus (BPEV, JN019858), cluster bean endornavirus (CBEV, MG764084), Cucumis melo endornavirus (CmEV, KT727022), Erysiphe cichoracearum endornavirus (EcEV, KT388110), Gremmeniella abietina type B RNA virus XL (GaRV-XL, YP\_529670.1),grapevine endophyte endornavirus (GEEV, JX678977), Helianthus annuus endornavirus 1 (HaEV1, MF362666), **Helicobasidium mompa endornavirus 1 (HmEV-1,** [AB218287.1](http://www.ncbi.nlm.nih.gov/nuccore/AB218287.1)**),** Hordeum vulgare endornavirus (HvEV, [KT721705.1](http://www.ncbi.nlm.nih.gov/nuccore/KT721705.1)), hot pepper endornavirus (HpEV, [KR080326](http://www.ncbi.nlm.nih.gov/nuccore/KR080326.1)), Lagenaria siceraria endornavirus (LsEV, [KF562072](http://www.ncbi.nlm.nih.gov/nuccore/KF562072.1)), **Oryza rufipogon endornavirus (OrEV,** YP\_438202.1**), Oryza sativa endornavirus (OsEV,** YP\_438200.1**), Persea americana endornavirus 1 (PaEV1,** YP\_005086952.1**),Phaseolus vulgaris endornavirus 1 (PvEV1,** [AB719397.1](http://www.ncbi.nlm.nih.gov/nuccore/AB719397.1)) Phaseolus vulgaris endornavirus 2 **(PvEV2,** AB719398.1**),**Phaseolus vulgaris endornavirus 3 **(PvEV3,** MG242064**),**Phytophthora endornavirus 1 (PEV1, YP\_241110.1), Rhizoctonia cerealis endornavirus 1 (RcEV1, KF311065), Rhizoctonia solani endornavirus 2 (RsEV2, KT823701), Rosellinia necatrix endornavirus 1 (RnEV1, LC076696), Sclerotinia minor endornavirus 1 (SmEV1, MG255170), Sclerotinia sclerotiorum endornavirus 1 (SsEV-1, KJ123645), Tuber aestivum endornavirus (TaEV, YP\_004123950), Vicia faba endornavirus (VfEV, YP\_438201), winged bean endornavirus (WBEV1, LC144945), yerba mate endornavirus (YmEV, KJ634409). The RdRP domain of grapevine leafroll-associated virus 1 (GLRaV-1, JQ023131), genus *Ampelovirus*, family *Closteroviridae*, was used as an outgroup. Positions of viruses proposed to be classified in new species are indicated by red font.