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:** | ***2017.012P*** | | | | (to be completed by ICTV officers) |
| **Short title: To create two new species in the family *Endornaviridae*** | | | | | |
| **Modules attached**  (Modules 1, 4 and either 2 or 3 are required. | | **1** **X 2 X 3  4 X** | | | |
| **Author(s):** | | | | | |
| **Sead Sabanadzovic**  **Rodrigo A Valverde**  **Ryo Okada** | | | | | |
| **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) | | | *Endornaviridae* 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: 2017.012P.N.v1.Endornaviridae\_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:** |
| **Edgar R.C., 2004**. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32: 1792-1797.  **Castresana J., 2000**. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol Biol Evol 17: 540-552.  **Hao F., Zhou Z., Wu M., Li G., 2017.** Molecular characterization of a novel endornavirus from the phytopathogenic fungus *Botrytis cinerea*. Arch Virol 162: 313-316.  **Huelsenbeck J.P., Ronquist F., 2001**. MRBAYES: Bayesian inference of phylogenetic trees*.* Bioinformatics 17: 754-755.  **Okada R., Kiyota E., Moriyama H., Fukuhara T., Valverde R.A., 2017**. Molecular and biological properties of an endornavirus infecting winged bean (*Psophacarpus tetragonolobus*). Virus Genes 53: 141-145. |

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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 family *Endornaviridae* (one in each of genera *Alphaendornavirus* and *Betaendornavirus*)**

Family *Endornaviridae* contains members infecting plants, fungi and oomycetes characterized by the lack of virus particles, efficient vertical transmission and, in most cases, absence of visible effects on the host. The family has been recently re-organized resulting in 22 recognized species grouped into two genera: *Alphaendornavirus* (18 species) and *Betaendornavirus* (4 species).

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

In this taxonomic proposal we propose the creation of two new species (one in the genus *Alphaendornavirus* and one in the genus *Betaendornavirus*) in order to classify two recently described viruses with properties resembling members of the family *Endornaviridae*.

**Winged bean endornavirus 1 (WBEV-1)**

A double-stranded RNA (dsRNA) of approximately 14.5-15 kbp, reminiscent of those associated with endornavirid infections, was isolated from asymptomatic winged bean (*Psophocarpus tetragonolobus*) plants of cv. Shikakumame and characterized (Okada et al., 2017). Sequence analyses confirmed that the dsRNA is a replicative form of a new virus, provisionally named winged bean endornavirus (WBEV-1). The complete sequence of WBEV-1 is 14,623 nt long and contains a single large ORF potentially coding for a polyprotein of an estimated molecular mass of 544 kDa with conserved motifs of putative methyltransferase (MTR), helicase (Hel-1), UDP-glycosyltransferase (UGT) and RNA-dependent RNA polymerase (RdRp) (Fig. 1). A comparison of amino acid sequences of the whole polyprotein with known endornaviruses revealed highest levels of identity (32-33%) with corresponding products of bell pepper endornavirus (BPEV), hot pepper endornavirus (HPEV) and Phaseolus vulgaris endornavirus 2 (PvEV-2), all of which share similar genome organization with WBEV-1. Similar to other plant-infecting endornaviruses, the dsRNA of WBEV-1 contains a site-specific nick at nt position 892. Phylogenetic analyses confirmed that WBEV-1 belongs to the same lineage with BPEV, HPEV and PvEV-2 within the genus *Alphaendornavirus* (Fig. 2). Results of a survey carried out as part of virus characterization suggest that WBEV-1 is common in winged beans (20 out of 32 tested PI lines and cultivars of *P. tetragonolobus* were infected). The virus was present in all 36 seedlings originated from seeds collected from an infected plant, indicating highly efficient vertical transmission. In conclusion, the genome organization, phylogenetic analyses and pair-wise sequence identities with recognized members of the genus *Alphaendornavirus* below the species demarcation threshold, indicate that WBEV-1 is a new member of that taxon and represents a new species named *Winged bean alphaendornavirus 1*.

**Botrytis cinerea endornavirus 1 (BcEV1)**

A novel endornavirus from the phytopathogenic fungus *Botrytis cinerea,* causal agent of grey mold disease on almost 1,500 different plants, has been recently characterized (Hao et al., 2017). The complete genome sequence of this virus, named Botrytis cinerea endornavirus 1 (BcEV1), was determined from the fungal strain HBtom-372. The BcEV1 genome is 11,557 nucleotides long, monocistronic and codes for a polyprotein of 3,787 amino acid residues. Results of hybridization experiments suggest lack of a site-specific nick that is present in some members of the family *Endornaviridae*. Searches in protein family databases revealed that the BcEV1-encoded polyprotein contains viral methyltransferase (MTR) domain, a cysteine-rich region (CRR), two putative viral helicase domains (DEXDc-like and Hel-1) and an RNA-dependent RNA polymerase (RdRp). Comparisons with genome products of recognized endornavirids showed that the BcEV1 polyprotein is most closely related to that of Sclerotinina sclerotiorum endornavirus 1 (SsEV1) as they share 39.1% common residues. Identities with other endornaviruses did not exceed 30%. In phylogenetic analysis, BcEV1 clustered with several endornaviruses infecting fungi belonging to the genus *Betaendornavirus*. BcEV1 was detected in 4.2% of 94 tested *B. cinerea* isolates collected from several provinces of central China (Hao et al., 2017). Based upon results of characterization of BcEV1 (genome size, organization, phylogeny, etc), and upon currently valid species demarcation criteria for the two genera in the family, we propose the creation of a new species, *Botrytis cinerea betaendornavirus 1*, in the genus *Betaendornavirus* (family *Endornaviridae*).

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**Figure 1.** Diagrammatic representation of the genome organization of WBEV-1 and BcEV with main nucleotide coordinates. The box represents the large, single ORF and lines represent untranslated regions (UTRs). Conserved domains present in the polyproteins expressed by the respective genomes are indicated in red: MTR = methyltransferase, CRR = cysteine-rich region, UGT = UDP-glycosyltransferase, DExH = DExH box, Hel-1 = helicase Superfamily 1, RdRp = RNA-dependent RNA polymerase. Figure is not to scale.

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**Figure 2.** Phylogenetic relationships of WBEV1 and BcEV1 with recognized members of the family *Endornaviridae*. The phylogenetic tree was reconstructed on amino acid sequences of virus RdRps using the Bayesian inference method implemented in the MrBayes program (v3.2.3) with Poisson model used for amino acid substitution and rate variation across sites fixed to "invgamma". Amino acid sequences were previously aligned with MUSCLE (Edgar, 2004) and refined with Gblocks (Castresana, 2000). Names, acronyms and GenBank accession numbers for viruses used to construct trees are: Alternaria brassicicola endornavirus 1 (AbEV-1, [KP239989.1](http://www.ncbi.nlm.nih.gov/nuccore/KP239989.1)), Basella alba endornavirus 1 (BaEV1, AB844264.1), Botrytis cinerea endornavirus 1 (BcEV1, KU923747.1), bell pepper endornavirus (BPEV, [JN019858.1](http://www.ncbi.nlm.nih.gov/nuccore/JN019858.1)), Cucumis melo endornavirus (CmEV, KT727022.1), Erysiphe cichoracearum endornavirus (EcEV, KT388110.1), Gremmeniella abietina type B RNA virus XL (GaRV-XL, YP\_529670.1),grapevine endophyte endornavirus (GEEV, [JX678977.1](http://www.ncbi.nlm.nih.gov/nuccore/JX678977.1)), **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, [KR080326.1](http://www.ncbi.nlm.nih.gov/nuccore/KR080326.1)), Lagenaria siceraria endornavirus (LsEV, [KF562072.1](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 (PvEV-1,** [AB719397.1](http://www.ncbi.nlm.nih.gov/nuccore/AB719397.1)) Phaseolus vulgaris endornavirus 2 **(**AB719398.1**),**Phytophthora endornavirus 1 (PEV1, YP\_241110.1), Rhizoctonia cerealis endornavirus 1 (RcEV1, KF311065.1), Sclerotinia sclerotiorum endornavirus 1 (SsEV-1, [KJ123645.1](http://www.ncbi.nlm.nih.gov/nuccore/KJ123645.1)), Tuber aestivum endornavirus (TaEV, YP\_004123950.1), Vicia faba endornavirus (VfEV, YP\_438201.1), winged bean endornavirus (WBEV1, LC144945.1), yerba mate endornavirus (YmEV, [KJ634409.1](http://www.ncbi.nlm.nih.gov/nuccore/KJ634409.1)). The closterovirus citrus tristeza virus (CTV, U16304.1) was used as an outgroup. Positions of WBEV1 and BcEV1 are indicated by asterisks.