This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). 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; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	***2015.022aP***	(to be completed by ICTV officers)

Short title: To create a new species in the genus *Endornavirus*.
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 10 are required)					
	1 ☒	2 ☒	3 ☐	4 ☐	5 ☐
	6 ☐	7 ☐	8 ☐	9 ☐	10 ☒

Author(s):

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Mike Pearson

Corresponding author with e-mail address:

Mahmoud Khalifa <mkha201@aucklanduni.ac.nz>

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 (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	R. Valverde, chair of the Endornavirus study group

ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV: June 16, 2015
Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	*2015.022aP*	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Endornavirus***
Subfamily:	***Unassigned***
Family:	***Endornaviridae***
Order:	***Unassigned***

Fill in all that apply.
- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Sclerotinia sclerotiorum endornavirus 1	11691	KJ123645

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Species demarcation criteria in the genus *Endornavirus*

According to the ninth report of ICTV (Fukuhara and Gibbs, 2012), species are distinguished on the basis of their host-range and sequence differences. Each recognized endornavirus species was isolated from a different host species. The genomic nucleotide sequences of different endornavirus species are only 30% to 75% identical.

***Sclerotinia sclerotiorum* endornavirus 1 (SsEV1), the proposed new species**

A recent study by Khalifa and Pearson (2014) has characterised a novel *Endornaviridae*-related RNA sequence from the New Zealand *Sclerotinia sclerotiorum* isolate 11691. In that publication, the name Sclerotinia sclerotiorum endornavirus 1 (SsEV1-11691) was adopted and the sequence deposited in the GenBank under accession number KJ123645. At the time of writing this proposal, this virus is listed in the GenBank as SsEV2 and a request to change it to SsEV1 has been made). Two other isolates have also been reported from *S. sclerotiorum* in China, SsEV1-isolate JZJL2 (accession no. KC852908) and the USA, SsEV1 isolate lactuca (accession no. KM923990). The sequences of these two viruses shared ~91% and ~81% nucleotide (nt) sequence identity respectively to the sequence of the virus isolated from *S. sclerotiorum* in New Zealand.

Properties of SsEV1-11691

SsEV1-11691 is 10513 nts in length and does not have a poly (A) tail at the 3´ untranslated region (UTR). SsEV1-11691 has a short 5´-UTR of 25 nts and a 108 nt long 3´-UTR, which ends in a poly (C) sequence stretch. It contains a single ORF that encodes a polyprotein of 3459 aa residues. The polyprotein encoded shares the highest sequence similarity with the unclassified virus from the ascomycete fungus *Gremmeniella abietina* [Gremmeniella abietina type B RNA virus XL1 (GaBRV-XL1). Like GaBRV-XL1, the coding strand of SsEV1 has no discontinuity (nick) at its 5´ end.

The genomes of endornaviruses encode single long polyproteins that include different domains. SsEV1-11691 has a genome structure similar to that of GaBRV-XL1 **(Figure 1)** and contains a viral methyltransferase (MTR) domain with the conserved motifs I, II, IV of the 'Sindbis-like' supergroup of ssRNA viruses, a putative DEXDc domain with the aa conserved motifs (I, II, III, V and VI) of the DExH box Hels, a viral Hel with the aa conserved motifs I-VI described for Hels of superfamilies 1 and 2 and an RdRp domain with the conserved motifs I-VIII identified using the CDD. The order in which MTR, Hel and RdRp are organised in SsEV1/11691 supports the hypothesis that endornaviruses and alpha-like viruses have a common ancestor (Gibbs et al., 2000) as described for *Phaseolus vulgaris endornavirus 2* (PvEV-2) by Okada et al. (2013). The polyprotein also contains a cysteine-rich region (CRR, C: 23%) and a Phytoreo_S7 (S7) domain **(Figure 1),** identified by analogy, at aa positions 778-838 and 2787-2890, respectively. The CRRs, analogous to that first reported by Hacker et al. (2005), contain multiple CxCC signatures with the most conserved signature being CxCCG (Tuomivirta et al., 2009).

The MTR domain shares 47.5% aa sequence identity with the corresponding domains of GaBRV-XL1, the most closely related putative endornavirus, **(Table 1).** Similarly, the "accessory" DExH box sequence shared 32.6% aa sequence identity with GaBRV-XL1 **(Table 1).** The viral Hel and RdRp domains shared aa sequence identities of 12.5-37.2% and 23.2-62.5% with endornaviruses recognized by the ICTV **(Table 1)**. SsEV1-11691 complete genome shares 26.6-51.3% nt sequence identities with endornaviruses **(Table 1)**. The nucleotide sequence identity of SsEV1-11691 with the most closely related endornavirus species (*Oryza sativa endornavirus*) was 36.7 %. The percentage sequence identity with GaBRV-XL1, an unclassified virus, was 51.3 % **(Table 1**).

A maximum-likelihood phylogenetic tree based on multiple alignments of the most conserved aa domain, the RdRp, showed that SsEV1-11691 is most closely related to the unclassified virus GaBRV-XL1 forming a separate cluster within the family **(Figure 2).** It is worth noting that SsEV1-11691 and GaBRV-XL1 were isolated from different ascomycetous hosts and shared similar features such as genome length, the absence of a positive-strand discontinuity and genome structure.

Results of virulence assays suggest that SsEV1/11691 have no obvious effects on the host phenotype and virulence.

As discussed above and detailed in the Khalifa and Pearson publication (2014), the biological properties, the genome structure and phylogenetic analysis and the low (36.7 %) nucleotide sequence identity with the closest endornavirus *Oryza sativa endornavirus* supports the proposition that Sclerotinia sclerotiorum endornavirus 1 (SsEV1) should be a new species in the family *Endornaviridae*.

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Fukuhara, T., Gibbs, M.J., 2012. Family *Endornaviridae*, in: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, London, UK, pp. 519-521.

Gibbs, M.J., Koga, R., Moriyama, H., Pfeiffer, P., Fukuhara, T., 2000. Phylogenetic analysis of some large double-stranded RNA replicons from plants suggests they evolved from a defective single-stranded RNA virus. J. Gen. Virol. 8, 227-233.

Hacker, C.V., Brasier, C.M., Buck, K.W., 2005. A double-stranded RNA from a *Phytophthora* species is related to the plant endornaviruses and contains a putative UDP glycosyltransferase gene. J. Gen. Virol. 86, 1561-1570.

Khalifa,M.E. and Pearson,M.N.(2014). Molecular characterisation of an endornavirus infecting the phytopathogen *Sclerotinia sclerotiorum*. Virus Research, 189, 303-309.

Okada, R., Yong, C.K., Valverde, R.A., Sabanadzovic, S., Aoki, N., Hotate, S., Kiyota, E., Moriyama, H., Fukuhara, T., 2013. Molecular characterization of two evolutionarily distinct endornaviruses co-infecting common bean (*Phaseolus vulgaris*). J. Gen. Virol. 94, 220-229.

Tuomivirta, T.T., Kaitera, J., Hantula, J., 2009. A novel putative virus of *Gremmeniella abietina* type B (Ascomycota: Helotiaceae) has a composite genome with endornavirus affinities. J. Gen. Virol. 90, 2299-2305.

Moriyama, H., Horiuchi, H., Nitta, T., Fukuhara, T., 1999. Unusual inheritance of evolutionarily-related double-stranded RNAs in interspecific hybrid between rice plants *Oryza sativa* and *Oryza rufipogon*. Plant Mol. Biol. 39, 1127-1136.

Moriyama, H., Nitta, T., Fukuhara, T., 1995. Double-stranded RNA in rice: A novel RNA replicon in plants. Mol. Gen. Genet. 248, 364-369.

Pfeiffer, P., 1998. Nucleotide sequence, genetic organization and expression strategy of the double-stranded RNA associated with the '447' cytoplasmic male sterility trait in *Vicia faba*. J. Gen. Virol. 79, 2349-2358.

Osaki, H., Nakamura, H., Sasaki, A., Matsumoto, N., Yoshida, K., 2006. An endornavirus from a hypovirulent strain of the violet root rot fungus, *Helicobasidium mompa*. Virus Res. 118, 143-149.

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

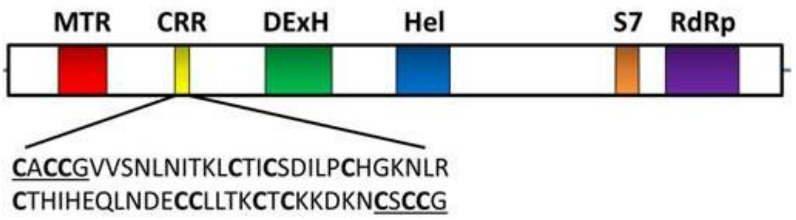

Figure 1. Schematic representation of the genome organisation of Sclerotinia sclerotiorum endornavirus 1 (SsEV1-11691). Conserved domains are represented by coloured boxes. The sequence of the cysteine-rich region (CRR, inset) contains two CxCCG signatures.

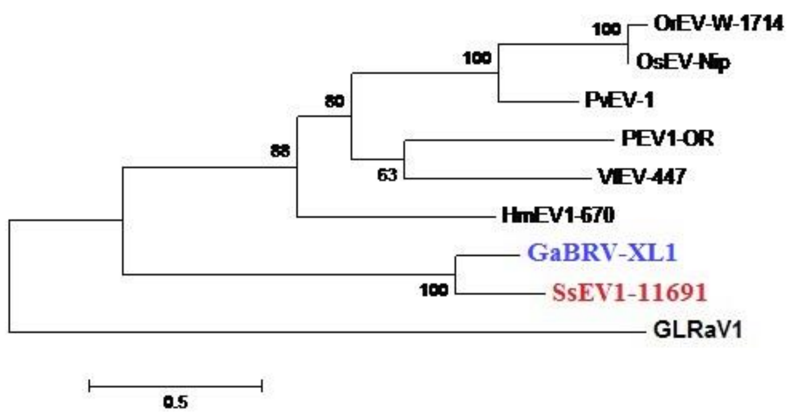

Figure 2. A maximum-likelihood phylogenetic trees constructed with the RNA-dependent RNA polymerase (RdRp) domains of Sclerotinia sclerotiorum endornavirus 1 (SsEV1-11691) and other endornaviruses (accepted species in **black**, proposed species in **red** and unclassified viruses in **blue**). The tree was constructed using LG with gamma-distributed site rates model of MEGA 6 software with bootstrapping analysis of 1000 replicates. The *Closterovirus Grapevine leafroll associated virus 1* (GLRaV1) was used as an outgroup. OrEV: *Oryza sativa endornavirus*; OrEV: *Oryza rufipogon endornavirus*; VfEV: *Vicia faba endornavirus*; PvEV-1: *Phaseolus vulgaris endornavirus 1*; HmEV1: *Helicobasidium mompa endornavirus 1*; PEV-1: *Phytophthora endornavirus 1* and GaBRV-XL1: Gremmeniella abietina type B RNA virus XL1.

Table 1 Sequence identities (%) between Sclerotinia sclerotiorum endornavirus 1 (SsEV1/11691) compared with endornavirus species (**black font**) and unclassified viruses (**blue font**) based on the multiple alignments of the complete nucleotide (nt) sequence and the amino acid (aa) sequences of different domains

Virus	Genome length (nts)	Acronym	Full sequence	Coding region					GenBank accession no.	Reference
			nt	MTR	DExH	Hel	S7	RdRp[a]		
Oryza sativa endornavirus	13952	OsEV/Nip	**36.7**	-	-	18.3	-	25.5	D32136	Moriyama et al., 1995
Oryza rufipogon endornavirus	13936	OrEV/W-1714	**36.4**	-	-	19.1	-	24.9	AB014344	Moriyama et al., 1999
Vicia faba endornavirus	17635	VfEV/447	**26.6**	-	-	12.5	-	25.6	AJ000929	Pfeiffer, 1998
Phaseolus vulgaris endornavirus 1	13908	PvEV-1	**30.9**	-	-	17.0	-	23.2	AB719397	Okada et al., 2013
Helicobasidium mompa endornavirus 1	16614	HmEV1-670	**28.5**	-	-	14.2	15.6	25.6	AB218287	Osaki et al., 2006
Gremmeniella abietina type B RNA virus XL1	10375	GaBRV-XL1	**51.3**	47.5	32.6	37.2	52.3	62.5	DQ399289	Tuomivirta et al., 2009
Phytophthora endornavirus 1	13883	PEV1-OR	**28.7**	-	-	13.2	-	24.6	AJ877914	Hacker et al., 2005

[a]: about 255 aa-long stretches of the RdRp conserved motifs were used.