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:	***2013.008a,bP***	(to be completed by ICTV officers)

Short title: To create a new species in the genus *Endornavirus* and change the name of an existing taxon.

(e.g. 6 new species in the genus *Zetavirus*)

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

Author(s) with e-mail address(es) of the proposer:

Valverde, R. A. (ravalve@lsu.edu)
Okada, R.
Sabanadzovic, S.
Moriyama, H.
Fukuhara, T.

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)	*Endornaviridae*

ICTV-EC or Study Group comments and response of the proposer:

None

Date first submitted to ICTV: June 2013
Date of this revision (if different to above):

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	***2013.008aP***	(assigned by ICTV officers)

To create one new species within:

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

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.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
***Phaseolus vulgaris endornavirus* 2**	AB719398

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

Currently, endornavirus species are distinguished on the basis of their host-range and sequence differences. Each newly recognized endornavirus species has been isolated from a different host species. The genomic nucleotide sequences of different endornavirus species ranges from 30% to 75% identity (Fukuhara & Gibbs, 2012).

The nucleotide sequence of a 630 bp dsRNA from a putative endornavirus isolated from Black Turtle Soup was published by Wakarchuk & Hamilton, (1990) (GenBank accession No. X16637). Based on the sequence and other properties of this dsRNA, the virus was placed in the genus *Endornavirus* and named *Phaseolus vulgaris endornavirus* (PvV) (Fukuhara *et al*., 2006). Fukuhara *et al*., (2006) and Segundo *et al*., (2008) reported partial sequences for PvV (GenBank accession numbers AB185245 and AM284175 respectively) isolated from common bean.

Recently, we have determined that the common bean (*Phaseolus vulgaris*) cultivar Black Turtle Soup is infected with two putative endornaviruses [(tentatively named *Phaseolus vulgaris* endornavirus 1 (PvEV-1) and *Phaseolus vulgaris* endornavirus 2 (PvEV-2)] with a genome size of 13,908 and 14,820 bp respectively (Okada et al., 2013). The dsRNAs of these viruses were clearly resolved after long runs in agarose gel electrophoresis and ethidium bromide staining (Fig. 1). Both PvEV-1 and PvEV-2 were transmitted to the progeny plants at rates close to 100%, a biological property typical of plant endornaviruses. After analyzing the sequence of these two putative endornaviruses, we concluded that they consisted of two distinct viruses; one identical to *Phaseolus vulgaris endornavirus* described by Wakarchuck & Hamilton and the other a putative new species of the genus *Endornavirus*.

Both PvEV-1 and PvEV-2 contain a single ORF in the coding strand of 4,496 and 4,851 codons, respectively. Comparison of amino acid (aa) sequences of the polyproteins encoded by

these ORFs revealed low levels of mutual identity and similarity (5% and 17%, respectively). A BLAST search (NCBI database) using deduced aa sequences from both ORFs encoded by PvEV-1 and PvEV-2 detected conserved domains of a putative RNA helicase-1 (Hel-1), UDP-glucose-glycosyltransferase (UGT) and an RNA-dependent RNA polymerase (RdRp). A putative viral methyltransferase (MTR) domain was only found in PvEV-2, whereas conserved putative capsular polysaccharide synthase (CPS)-like domain was only present in PvEV-1 (Fig. 2).

A unique molecular feature of the member of the family *Endornaviridae* is the presence of a site-specific nick in the 5' region of the coding (plus) strand RNA molecule. Sequence analyses of multiple RACE-generated clones indicated the presence of a nick in the coding strand at nt 1,111 for PvEV-1 and nt 881 for PvEV-2.

Subcellular fractionation was performed to determine the localization of PvEV-1 and PvEV-2 in the host cells. PvEV-1 was associated with the microsomal fraction while PvEV-2 was mainly associated with the crude chloroplast and mitochondrial fractions. These results suggest different intracellular localization for PvEV-1 and PvEV-2. Although, two common bean cultivars were found to be infected only with PvEV-2, the viruses were found together in most virus-infected common bean cultivars.

Phylogenetic analyses of the putative Hel-1, UGT and RdRp showed that these two dsRNA viruses clustered with other members of the family *Endornaviridae* (a phylogenetic tree using the viral RdRp is shown in Fig.3). While PvEV-1 is closely related to *Oryza sativa endornavirus*, PvEV-2 appears to be a close relative of *Bell pepper endornavirus*.

The genome organizations and nucleotide sequences of PvEV-1 and PvEV-2 strongly support the notion that they are distinct endornavirus species. Therefore, we propose that both viruses be classified into the genus *Endornavirus* of the family *Endornaviridae* and propose the names of *Phaseolus vulgaris endornavirus* 1 for the 13,908 bp virus and *Phaseolus vulgaris endornavirus* 2 for the 14,820 bp virus.

MODULE 8: **NON-STANDARD**

Template for any proposal not covered by modules 2-7. This includes proposals to change the name of existing taxa (but note that stability of nomenclature is encouraged wherever possible).

non-standard proposal

Code	***2013.008bP***	(assigned by ICTV officers)

Title of proposal: To change the name of the existing taxon *Phaseolus vulgaris endornavirus* to *Phaseolus vulgaris endornavirus* 1.

Text of proposal:

The nucleotide sequence of a 630 bp dsRNA from a putative endornavirus isolated from the common bean cultivar Black Turtle Soup was published by Wakarchuk & Hamilton, (1990). Based on the sequence and other properties of this dsRNA, the virus was placed in the genus *Endornavirus* and named *Phaseolus vulgaris endornavirus* (PvV) (Fukuhara *et al*., 2006; Fukuhara & Gibbs, 2012).

We have determined that the common bean (*Phaseolus vulgaris*) cultivar Black Turtle Soup is infected with two distinct endornaviruses, *Phaseolus vulgaris* endornavirus 1 (GenBank sequence accession No. AB719398) and *Phaseolus vulgaris* endornavirus 2 (GenBank sequence accession No. AB719397), with a genome size of 13,908 and 14,820 bp respectively. After analyzing their genome sequences and compared with sequences in the GenBank, we determined that the partial sequences of the genome of PvV reported by Wakarchuk & Hamilton (1990) were derived from *Phaseolus vulgaris* endornavirus 1. Therefore, we propose that *Phaseolus vulgaris endornavirus* should be renamed *Phaseolus vulgaris* endornavirus 1 (PvEV-1) in order to clarify the nomenclature of these two endornaviruses co-infecting the same host. We are also proposing that *Phaseolus vulgaris* endornavirus 2 (PvEV-2) as a new member of the genus *Endornavirus*.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Fukuhara, T. & Gibbs, M. J. 2012. Family Endornaviridae. In Virus Taxonomy: Classification and Nomenclature of Viruses Ninth Report of the International Committee on Taxonomy of Viruses. Edited by Andrew M.Q. King, Michael J. Adams, Eric B. Carstens, and Elliot J. Lefkowitz. pp 519-521. Elsevier/Academic Press.

Fukuhara, T., Koga, R., Aoki, N., Yuki, C., Yamamoto, N., Oyama, N., Udagawa, T., Horiuchi, H., Miyazaki, S. & other authors (2006). The wide distribution of endornaviruses, large double-stranded RNA replicons with plasmid-like properties. *Arch Virol* 151: 995-1002.

Okada, R., Young, 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.

Segundo, E., Carmona, M. P. Sáez, E., Velasco, L., Martín, G., Ruiz, L., Janssen, D., & Cuadrado, I. M. (2008). Occurrence and incidence of viruses infecting green beans in south-eastern Spain. *Eur J Pl Pathol* 122: 579-591.

Wakarchuk, D. A. & Hamilton, R. I. (1990). Partial nucleotide sequence from enigmatic dsRNAs in Phaseolus vulgaris. *Plant Mol Biol* 14: 637-639.

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.

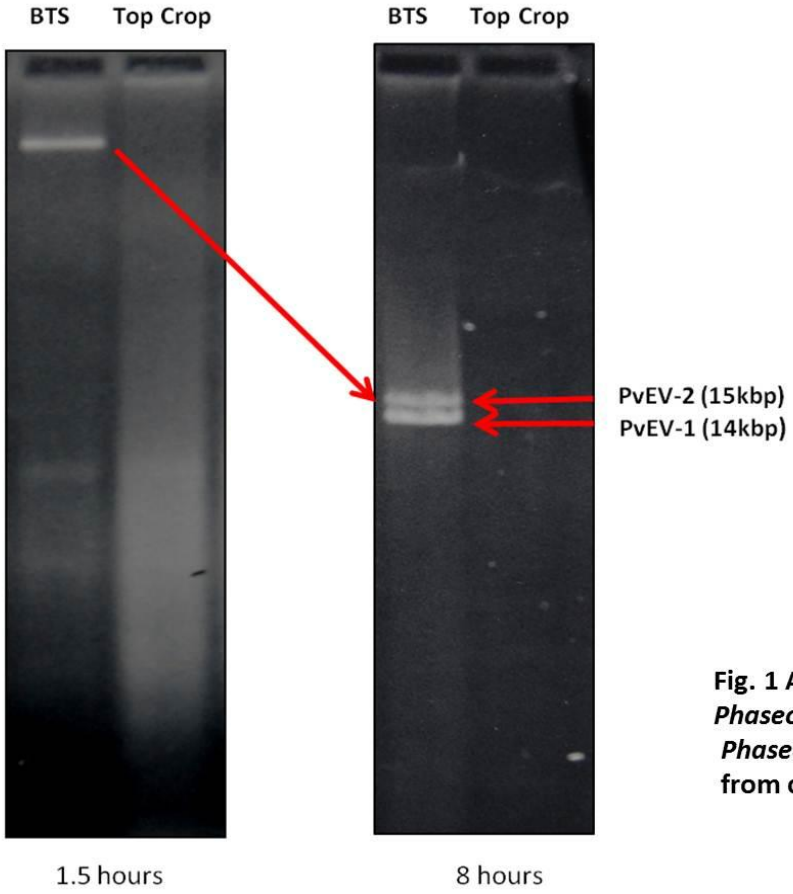

Fig. 1 Agarose gel electrophoresis of dsRNAs of *Phaseolus vulgaris* endornavirus 1 and *Phaseolus vulgaris* endornavirus 2 extracted from common bean Black Turtle Soup (BTS).

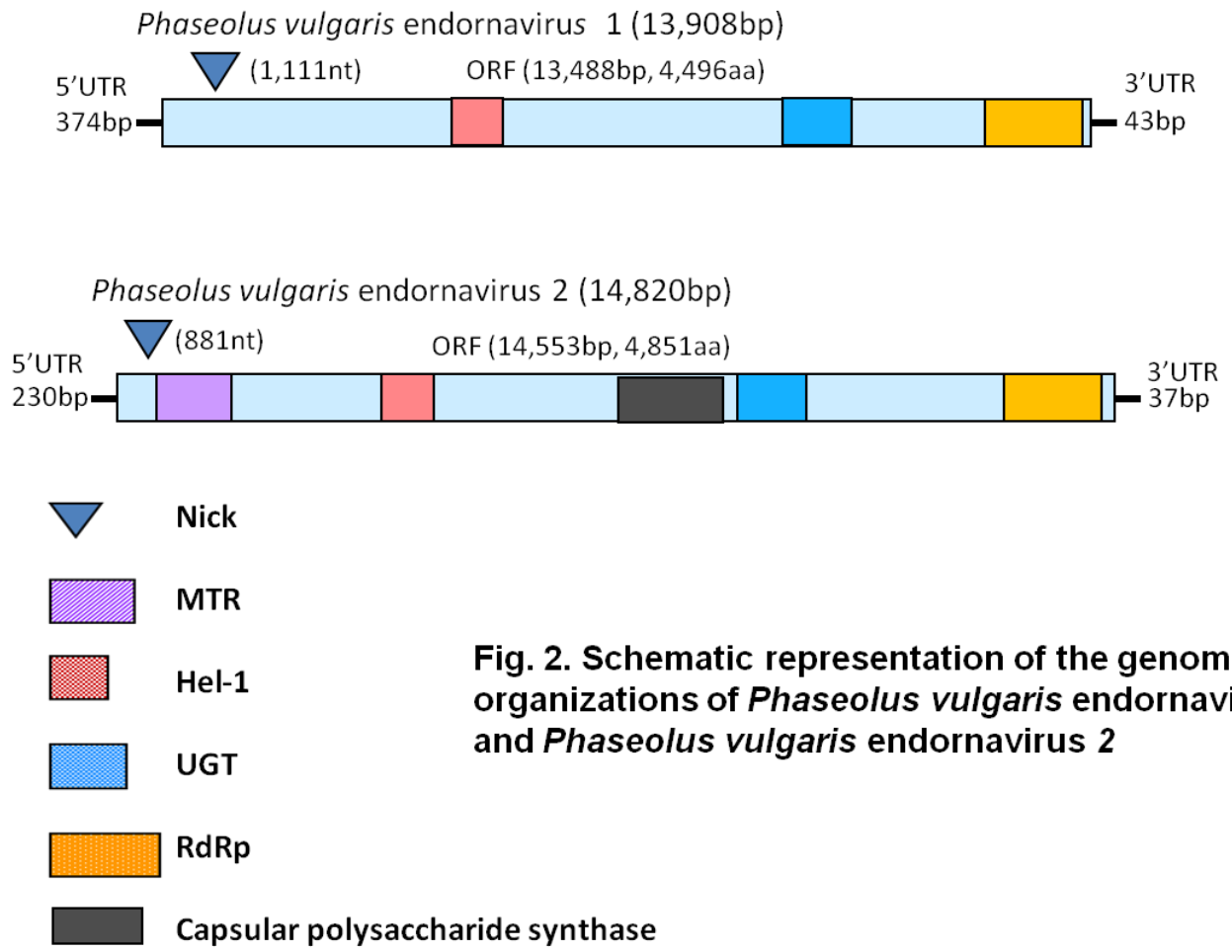

Fig. 2. Schematic representation of the genome organizations of *Phaseolus vulgaris* endornavirus 1 and *Phaseolus vulgaris* endornavirus *2*

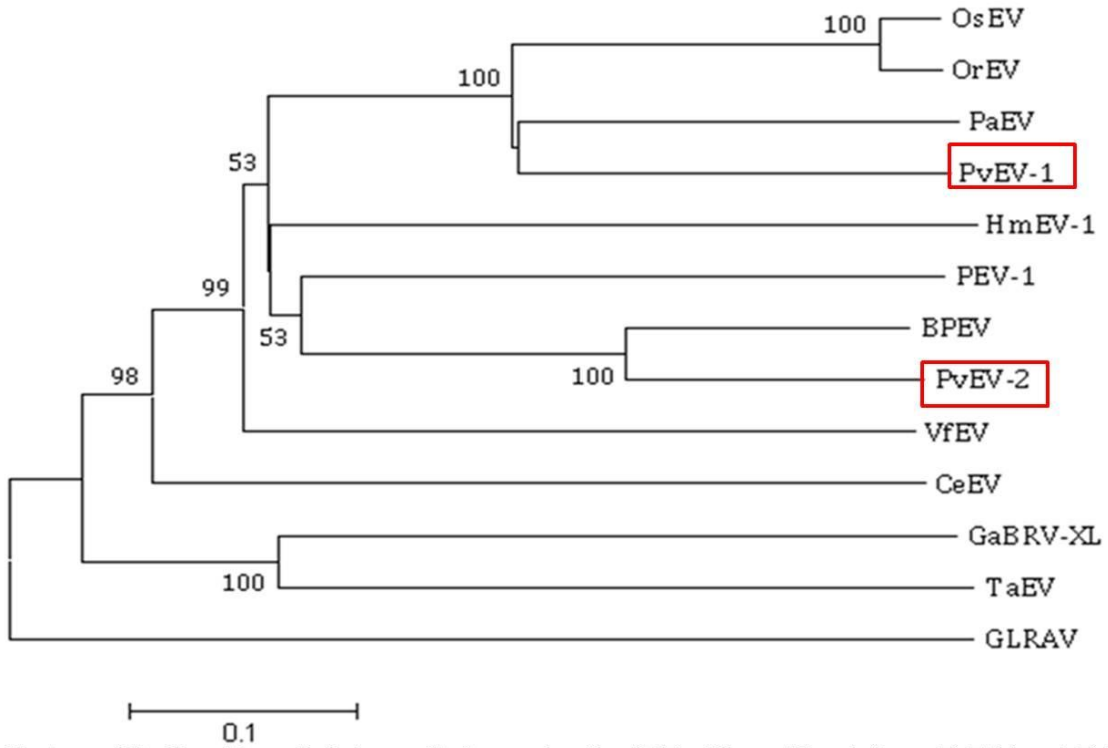

Fig. 3. Maximum Likelihood-based phylogenetic tree using the RdRp. Virus abbreviations: PvEV-1 and PvEV-2, *Phaseolus vulgaris* endornavirus 1 and 2 respectively; OsEV, *Oryza sativa endornavirus*; OrEV, *Oryza rufipogon endornavirus;* PaEV, *Persea americana* endornavirus; HmEV-1, *Helicobasidium mompa endornavirus*; BPEV, *Bell pepper endornavirus*; PEV-1, *Phytophthora endornavirus* 1; GaBRV-XL, *Gremmeniella abietina* type B RNA virus XL1; VfEV, *Vicia faba endornavirus*; CeEV1, *Chalara elegans* endornavirus; TaEV, *Tuber aestivum* endornavirus and GLRaV, *Grapevine leafroll-associated virus* 2 (used as an outgroup).