## **Template for Taxonomic Proposal to the ICTV Executive Committee To create a new Family**

Code <sup>†</sup> <b>2006.019P.04</b>	To create a new unassigned family*
Code <sup>†</sup> <b>2006.020P.04</b>	To name the new family* <i>Endornaviridae</i>
Code <sup>†</sup>	To designate the following genera as part of the new family*:
	Endornavirus
•	te responding arguments for each genus created in the family address(es) of the Taxonomic Proposal
Dr. T. Fukuhara, Tokyo, J Dr. M.J. Gibbs, Canberra,	

### **Old Taxonomic Order**

Order Family Genus Endornavirus Type Species Oryza sativa endornavirus (OSV) Species in the Genus Oryza rufipogon endornavirus (ORV) Oryza sativa endornavirus (OSV) Phaseolus vulgaris endornavirus (PVuV) Vicia faba endornavirus (VFV)

Tentative Species in the Genus

#### New Taxonomic Order

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**Tentative Species in the Genus** 

#### *Gremmeniella abietina* type B RNA virus XL1 (GaRV-XL1) *Bell pepper endornavirus*, (BpEV)

Unassigned Species in the family

#### **ICTV-EC** comments and response of the SG

#### Argumentation to create a new family:

Linear double-stranded RNAs (dsRNAs) have frequently been found in various healthy plants. Large dsRNAs (about 14 kbp) have been detected in many symptomless cultivars of rice (*Oryza sativa*). This dsRNA occurs in every tissue and at every developmental stage, and is maintained at an almost constant concentration (100 copies/cell) in most tissues. Although the dsRNA is found in the cytoplasm of host cells, it is transmitted very efficiently (> 98%) to progeny plants via pollen and ova. These properties differ from those of conventional viruses. Large dsRNAs have also been found in many other crops, but only three of these dsRNAs, from cultivated rice, wild rice (*O. rufipogon*) and broad bean (*Vicia faba*), have been sequenced completely. All three dsRNAs encode a single long open reading frame (ORF), in which conserved motifs for RNA-dependent RNA polymerase (RdRp) and RNA helicase (Hel) are found. These large dsRNAs are RNA replicons that can replicate independently of their host genome. Phylogenetic analyses of these three dsRNAs indicate that they share a common ancestor with the alpha-like supergroup of single-stranded RNA (ssRNA) viruses. We proposed that these large dsRNA replicons be recognized as members of a new virus genus *Endornavirus*, and ICTV accepted the new genus *Endornavirus* for the eighth report.

The partial nucleotide sequences of the RdRp regions from the large dsRNAs (about 14 kbp) isolated from barley (*Hordeum vulgare*), kidney bean (*Phaseolus vulgaris*), melon (*Cucumis melo*), bottle gourd (*Lagenaria siceraria*), Malabar spinach (*Basella alba*), seagrass (*Zostera marina*), and the strain V1369 of the violet root rot fungus (*Helicobasidium mompa*) have been published recently, and their sequences are significantly similar to those of endornaviruses (Fukuhara *et al.*, 2006). The complete nucleotide sequence of the 13.9 kbp dsRNA isolated from a *Phytophthora* species has also been published recently (Hacker et al., 2005). *Phytophthora* is classified in a protist group known as the Stramenopiles. Phylogenetic analyses indicate that this dsRNA should be classified as a member of the genus *Endornavirus*, and has been tentatively named *Phytophthora* endornavirus 1 (PEV1). Furthermore, the complete nucleotide sequence of the 16.7 kbp L1 dsRNA from the strain V670 of the violet root rot fungus (*Helicobasidium mompa*) has been reported (Osaki et al., 2006). Phylogenetic analyses indicates that this fungal dsRNA is also an endornavirus with the suggested name *Helicobasidium mompa* 

endornavirus 1-670 (HmEV1-670). Therefore, there is a growing list of new dsRNA viruses in the genus *Endornavirus*, which are distributed over the plant, protist and fungal kingdoms.

Because some recently published sequences of new endornaviruses are available, we carried out phylogenetic analyses using amino acid (aa) sequences of the entire RdRp regions (about 470 aa residues) of the three new endornaviruses (*Lagenaria siceraria endornavirus* (LsEV), PEV1 and HmEV1-670), the four known endornaviruses (OsEV, OrEV, PvEV and VfEV), and 16 ssRNA viruses of the alpha-like supergroup (including closteroviruses, tobamoviruses, bromoviruses etc), by ClustalX and MEGA2 (Molecular Evolutionary Genetics Analysis). The resulting neighbor-joining (NJ) tree shows that three new endornaviruses formed a monophyletic group with the known endornaviruses, which was supported by a high bootstrap probability (100%, Fig. 1A). Furthermore, we carried out phylogenetic analyses with additional five partial sequences of RdRp regions of probable new endornaviruses (BaEV, CmEV, HvEV, ZmEV and HmEV1369). Although the analyzed RdRp regions were short (about 90 aa), the phylogenetic tree obtained was essentially the same as the tree in Fig. 1A, and all twelve sequences formed a monophyletic group (Fig. 1B). These twelve endornaviruses have no homology to recognized dsRNA and ssRNA viruses and form a single independent virus group.

Comparison of the genome organizations of four endornaviruses (OsEV, PEV1, VfEV and HmEV1-670) indicates that they share a common unique structure, though their hosts are distributed over the plant, protist and fungal kingdoms (Fig. 2). They encode a single unusual long ORF, in which conserved motifs for RdRp and Hel are found in the same region. They also contain a site-specific nick in the 5' region of the coding strand, which divides the long ORF. These two molecular features, the single long ORF and the site-specific nick have never been found in known RNA viruses. Therefore, we propose that the endornaviruses should be assigned to a separate family, for which we suggest the name *Endornaviridae*.

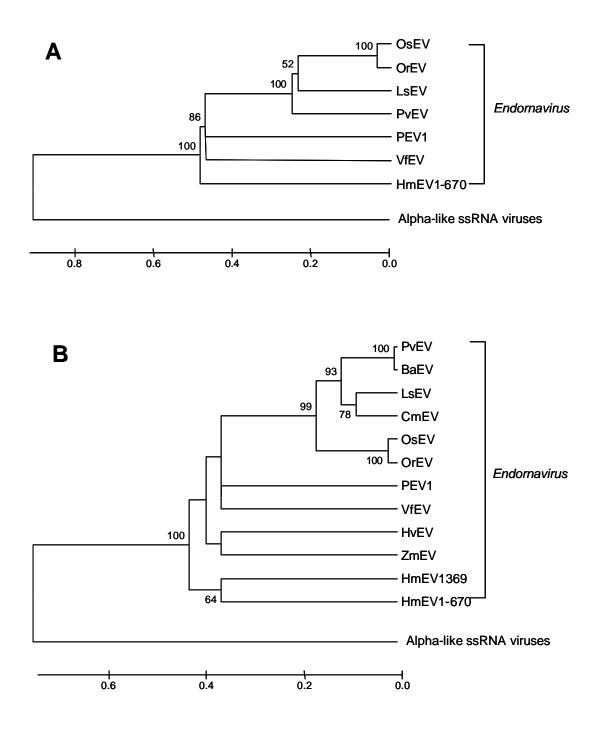


Fig. 1. Phylogenetic position of endornaviruses. (A) About 470 aa of RdRp regions of seven endornaviruses and 16 alpha-like ssRNA viruses and (B) about 90 aa of the RdRp regions of twelve endornaviruses and 16 alpha-like ssRNA viruses were analyzed by the NJ method (MEGA ver. 2.1). A bootstrap test was performed with 100 resamplings.

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Fig. 2. Comparison of genome organizations of four endornaviruses: *Oryza sativa endornavirus* (OsEV), *Phytophthora endornavirus* 1 (PEV1), *Vicia faba endornavirus* (VfEV), and *Helicobasidium mompa endornavirus* 1-670 (HmEV1-670).

# Origin of the proposed family name

These dsRNAs have been called 'endogenous dsRNAs'. Endo, from Greek: within, and *RNA* 

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#### Annexes: