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:	***2009.009a,bP***	(to be completed by ICTV officers)

Short title: create 1 new species in the genus Tobamovirus, family Virgaviridae
(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:

Zhenchen Zhang, zhangzhenchen@126.com
Lifang Zhang, amelia-lifang@hotmail.com

Has this proposal has been seen and agreed by the relevant study group(s)? Please select answer in the box on the right	**Yes**

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

Date first submitted to ICTV: 2009-04-21
Date of this revision (if different to above):

MODULE 2: **NEW SPECIES**

Part (a) to create and name one or more new species.
If more than one, they should be a group of related species belonging to the same genus (see Part b)

Code	***2009.009aP***	(assigned by ICTV officers)

To create 1 new species with the name(s):

Rehmannia mosaic virus

Part (b) assigning new species to higher taxa
All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family.

Code	***2009.009bP***	(assigned by ICTV officers)

To assign the species listed in section 2(a) as follows:

Genus:	***Tobamovirus***
Subfamily:	
Family:	***Virgaviridae*** (awaiting ratification)
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.

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.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Species demarcation criteria in the genus *Tobamovirus* are:
- Sequence similarity; less than 10% overall nt sequence difference is considered to characterize strains of the same species, although most of the sequenced species have considerably less than 90% sequence identity,
- Host range; however many of these viruses have wider and more overlapping host ranges in experimental rather than natural situations,
- Antigenic relationships between the CPs.

Rehmannia mosaic virus

The scrophulariaceous species rehmannia (*Rehmannia glutinosa* Libosch) is an important herbaceous medicinal plant in China [1]. Viral diseases cause significant losses of both yield and quality of this plant. However, the viruses causing these diseases are still not fully characterized [2]. We have identified an isolate similar to TMV, eliciting systemic mosaic symptoms, from Rehmannia in Henan Province, China but TMV itself is not known to infect members of this plant family. We have temporarily named this isolate Rehmannia mosaic virus (ReMV). The biological characteristics of ReMV were detected. Primary investigations have shown that this isolate could infect 10 plant species in 4 families. It could cause necrotic lesions on *Nicotiana glutinosa*, *N. rustica, Datura stramonium*, *Chenopodium quinoa*, *Chenopodium amaranticolor*, *Tetragonia expansa*; induce system mosaic on *N. benthamiana*, *Nicotiana tabacum* cv. K326, *Lycopersicon esculentum* and *Brassica pekinensis.* TMV does not infect

Brassica species.

The ReMV isolate was mechanically inoculated onto *N. glutinosa* in the greenhouse. After two successive single lesion isolations, the virus was propagated in *N. tabacum* cv. Yunyan 87. Virus purification was conducted according to a previously described method [3]. Purified virus preparation showed A_{260}/A_{280} ratio of ca. 1.47, 10^{-5}- 10^{-6} dilution end point, 90□-95□ thermal inactivation point and retained its infectivity after being kept at 20 °C for over 60 days. Electron micrograph of puified virus preparation revealed rod shaped particles of ReMV (Fig. 1).

We also investigated the complete genome sequence of this isolate by RT-PCR and RACE [4]. The ReMV RNA genome (GenBank accession number EF375551) is composed of 6,395 nts, from which four open reading frames (ORFs) are predicted. The 5′ and 3′ UTR regions contain 71 and 204 nts, respectively. The first ORF, encoding a 126-kDa protein, begins at nt 72 and ends at nt 3422 with a TAG stop codon and a downstream CAATTA sequence (nt 3,423–3,428). Based on previous studies on TMV and related tobamoviruses [5], it is likely that this TAG stop codon and the downstream CAATTA sequence are recognized by the GΨA anticodon, leading to the readthrough of this codon and the production of a 183-kDa protein. Consequently, the ORF encoding the 183-kDa protein begins at nt 72 and ends at nt 4,922. The third ORF (coding for a 30-kDa protein) starts at nt 4,906 and ends at nt 5,709, and the fourth ORF spans nts 5,712–6,191. The 30- and 17.5-kDa protein ORFs are separated by two nucleotides that possess the characteristics of the subgroup I tobamoviruses [6].

The ReMV genome exhibits the highest nt identity to that of TMV-type (84%). In line with this finding, nucleotide and/or amino acid sequence comparisons reveal that the 5′ and 3′ UTRs and the four ORFs of ReMV are also more similar to the corresponding regions in TMV isolates than to those in other tobamoviruses or isolates (Table 1). The phylogenetic relationships among ReMV and other tobamoviruses are shown in Fig. 2. From the patterns of phylogenetic clustering depicted in Fig. 2, it is clear that the RNA genome of ReMV is more closely related to those of TMV-U1 or TMV-type than other tobamoviruses. In accordance with this observation, closer relationships are also evident between the 183-, 30- and 17.5-kDa proteins of ReMV and their counterparts encoded by TMV-U1 or TMV-type.

The eighth report of the International Committee on the taxonomy of viruses (ICTV) states that if the differences between two tobamoviruses are less than 10% of the overall nt sequence, they should be characterized as strains of the same species. Because the minimal nt difference between ReMV and TMV-type, with which ReMV is generally more closely related, was 16%, ReMV may thus represent a new member of the genus *Tobamovirus*. Gibbs et al. [7] compared the complete genomic sequences of 48 tobamoviruses and suggested that a short nucleotide motif in the polymerase gene, the ‘‘4404–50 motif’’, be used to distinguish between different tobamoviruses. The ‘‘4404–50 motif’’ consists of conserved genus-specific sites intercalated with variable sites that provide species-specific nucleotide combinations [7]. From Table 2, it is apparent that the ‘‘4404–50 motif’’ of ReMV is identical to the conserved genus-specific sites of tobamoviruses, supporting that ReMV is a tobamovirus. There are 5 nt differences between ReMV and the TMV-specific motif, and 12 nt differences between ReMV and the ToMV-specific motif, indicating that the ReMV ‘‘4404–50 motif’’ is different from the corresponding elements in all known TMV and ToMV isolates. This result provides further evidence that ReMV is a new member of genus *Tobamovirus*.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

[1] Zhang ZC, Qiao Q, Jin XL, Wang YJ (2004) The techniques and application of virus-free Rehmannia glutinosa (in Chinese). Acta Phytophylacica Sin 31(4):342–346
[2] Zhang ZC, Zhang LF, Qiao Q, Wang YJ, Jin XL (2004) Identification of the viral pathogens of Rehmannia glutinosa disease in Henan Province (in Chinese). Acta Phytopathol Sin 34(5): 395–399
[3] Gooding JV, Heoert TT (1967) A simple technique for purification of tobacco mosaic virus in large quantities. Phytopathology 57(11):1258
[4] Z. C. Zhang C. Y. Lei L. F. Zhang X. X. Yang R. Chen D. S. Zhang (2008). The complete nucleotide sequence of a novel Tobamovirus, Rehmannia mosaic virus. Arch Virol (153): 595-599.
[5] Yang G, Liu XG, Qiu BB (2000) Complete nucleotide sequences and genome structure of two Chinese tobacco mosaic virus isolates deduced from full-length infectious cDNA clones (in Chinese). Chin J Biotechnol 16(4):437–442
[6] Lartey RT, Voss TC, Melcher U (1996) Tobamovirus evolution: gene overlaps, recombination, and taxonomic implications. Mol Biol Evol 13(10):1327–1338
[7] Gibbs AJ, Armstrong JS, Gibbs MJ (2004) A type of nucleotide motif that distinguishes Tobamovirus species more efficiently than nucleotide signatures. Arch Virol 149:1941–1954

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.

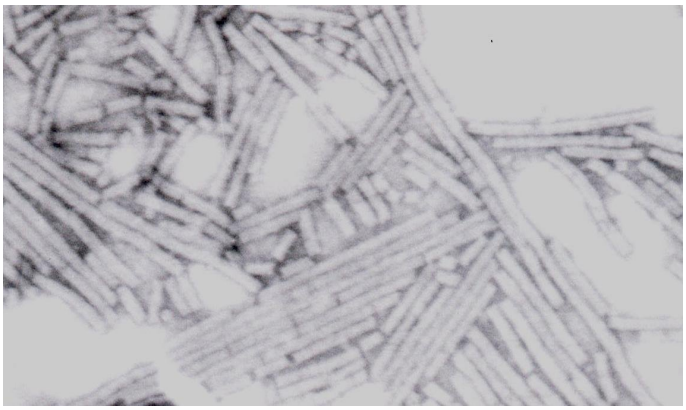

Figure 1. Electron micrograph of purified ReMV particles visualized by negative staining with 1% w/v phosphotungstic acid (PTA).

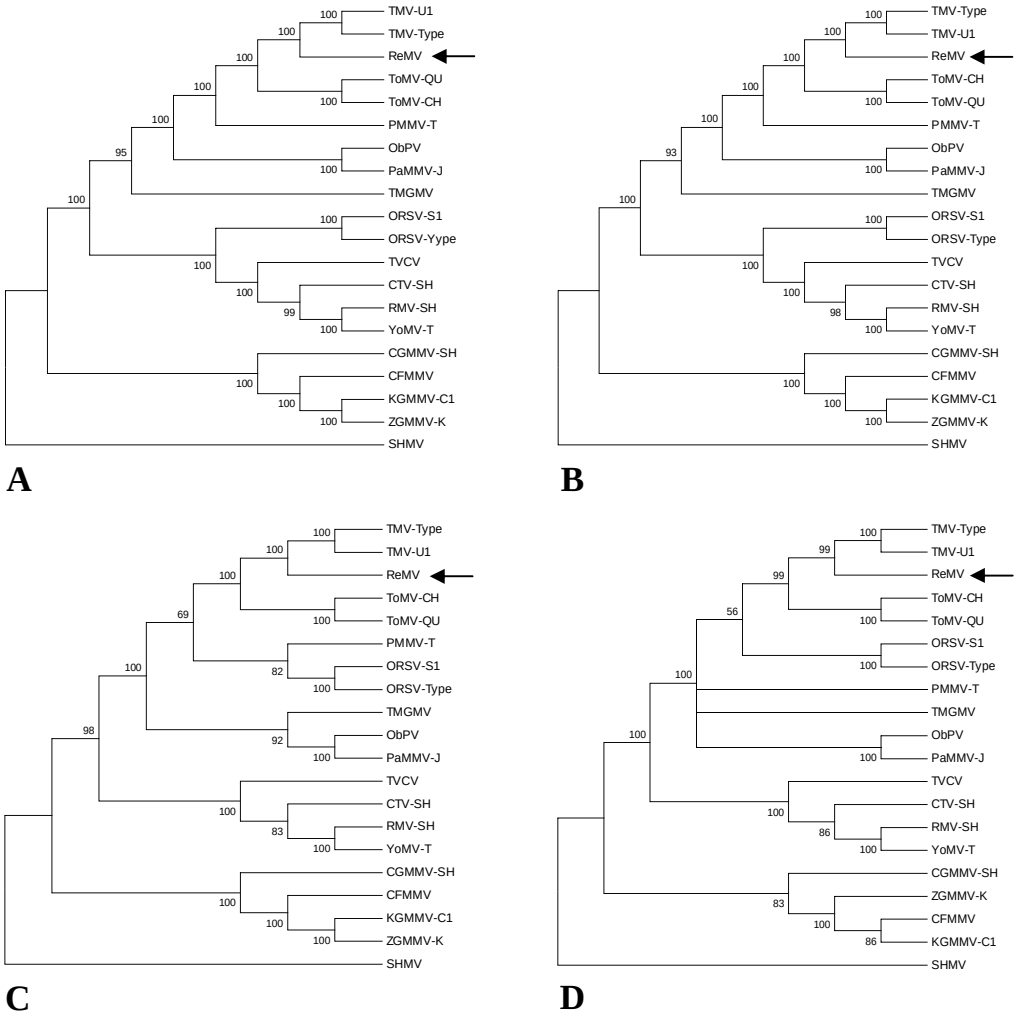

Figure 2. Investigations of the relationships of ReMV (indicated by arrow) to previously reported tobamoviruses based on phylogenetic analyses using the complete genomic nt sequences (including those of 5′- and 3′ UTRs) (A) or the deduced amino acids sequences of the 183 kDa (B), 30 kDa (C), and 17.5 kDa (D) proteins. Multiple alignments of the amino acid sequences were carried out using the ClustalW program at the EBI website (http://www.ebi.ac.uk/clustalw/). The phylogenetic trees were constructed at the MEGA browser (http://www.megasoftware.net/). The trees displayed were developed using the Neighbor-joining program. Identical trees were obtained by using other algorithms (i.e., minimum evolution, maximum parsimony). The number beside each node indicates bootstrap value, which was obtained based on 500 replications.

Table 1. Nucleotide and amino acid sequence identities among ReMV and previously reported tobamoviruses

Virus	126 kDa		183 kDa		30 kDa		17.5 kDa		5´ UTR	3´ UTR	Full length
	nt	aa	nt	aa	nt	aa	nt	aa	nt	nt	nt
TMV-Type	83	93	84	94	81	86	86	95	89	95	84
TMV-U1	82	93	83	94	80	85	85	94	92	94	83
ToMV-QU	78	90	79	90	72	76	76	81	83	80	78
ToMV-CH	79	90	80	91	70	76	76	81	79	80	78
PMMV-T	68	73	69	75	64	61	66	72	70	61	68
ObPV	63	67	64	69	55	51	59	59	59	40	61
PaMMV-J	63	66	64	69	54	50	60	60	63	36	61
TMGMV	61	63	63	66	56	48	65	72	59	54	63
ORSV-S1	56	34	58	62	53	52	64	70	39	21	57
ORSV-Type	55	32	58	60	52	51	63	69	38	27	57
RMV-SH	58	59	60	63	46	34	48	45	56	36	57
YoMV-T	58	59	60	63	46	34	48	45	55	36	57
CTV-SH	58	57	60	62	46	35	49	44	51	36	56
TVCV	58	58	61	63	45	33	51	46	52	37	57
SHMV	47	39	49	43	36	19	45	40	42	39	46
KGMMV-C1	45	38	48	43	39	26	41	32	34	44	46
ZGMMV-K	45	39	48	63	38	27	42	33	32	46	45
CFMMV	45	38	48	43	39	25	42	33	39	51	45
CGMMV-SH	44	39	48	45	40	25	43	34	52	51	45

Table 2. Comparisons of the "4404-50 motif" in ReMV, TMV, ToMV and *Tobamovirus*

	4404	4450
ReMV	**GG**T**GA**C**GT**T**AC**C**AC**G**TT**C**AT**T**GG**G**AA**C**AC**A**GT**G**AT**C**AT**C**GC**T**GC**A**TG**	
TMV specific motif	**GG**G.**A**.**GT**C**AC**G**AC**.**TT**C**AT**T**GG**A**AA**C**AC**.G**T**G**AT**C**AT**T**GC**T**GC**.**TG**	
ToMV specific motif	**GG**T**GA**T**GT**T**AC**A**AC**T**TT**T**AT**C**GG**T**AA**T**AC**C**GT**C**AT**C**AT**T**GC**TT**C**G**TG**	
Tobamovirus	**GG..A.GT.AC.AC.TT.AT.GG.AA.AC..T.AT.AT.GC..C.TG**	